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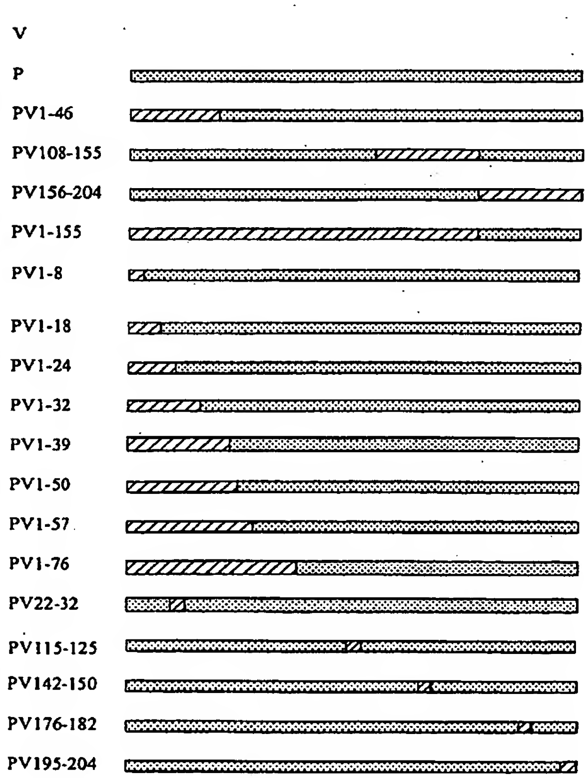

(57) Abstract: Disclosed are recombinant hybrid proteins having at least one antigenic peptide sequence introduced into a scaffold protein that retain a native conformation. Also disclosed are recombinant nucleic acids and vectors encoding the hybrid proteins. The hybrid proteins retain immunogenicity but exhibit reduced allergenicity. The hybrid proteins are therefore particulary useful for therapeutic treatment of allergy.

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RECOMBINANT HYBRID ALLERGEN CONSTRUCTS WITH REDUCED ALLERGENICITY THAT RETAIN IMMUNOGENICITY OF THE NATURAL ALLERGEN

This application claims priority under 35 U.S.C. § 119 (e) of U.S. Provisional Application Serial No. 60/272,818, filed March 2, 2001, which is hereby incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention is directed to recombinant hybrid proteins having native conformation and containing at least one antigenic peptide sequence introduced into a scaffold protein. The invention is further directed to recombinant nucleic acids and vectors encoding the recombinant vespid hybrid proteins and cells containing the recombinant vectors. Such recombinant hybrid proteins are useful for eliciting an immune response without eliciting an allergenic response, and are therefore particularly useful for therapeutic treatment of allergy.

BACKGROUND OF THE INVENTION

Genetically predisposed individuals become sensitized (allergic) to antigens originating from a variety of environmental sources, to the allergens of which the individuals are exposed. The allergic reaction occurs when a previously sensitized individual is re-exposed to the same or a homologous allergen. Allergic responses range from hay fever, rhinoconductivitis, rhinitis and asthma to systemic anaphylaxis and death in response to, e.g.,

bee or hornet sting or insect bite. The reaction is immediate and can be caused by a variety of allergens such as compounds originating from grasses, trees, weeds, insects, food, drugs, chemicals and perfumes.

Biochemical Aspects of Allergens

Insect sting allergy to bees and vespids is of common occurrence. The vespids include hornets, yellow jackets and wasps (Golden *et al.*, 1989, Am. Med. Assoc. 262:240). Susceptible people can be sensitized on exposure to minute amounts of venom proteins; as little as 2-10 μg of protein is injected into the skin on a single sting by a vespid (Hoffman and Jacobson, 1984, Ann. Allergy. 52:276).

There are many species of hornets (genus Dolichovespula), yellowjackets (genus Vespula) and wasp (genus Polistes) in North America (Akre *et al.*, 1980, "Yellowjackets of America North of Mexico," Agriculture Handbook No. 552, US Department of Agriculture). The vespids have similar venom compositions (King *et al.*, 1978, Biochemistry 17:5165; King *et al.*, 1983, Mol. Immunol. 20:297; King *et al.*, 1984, Arch. Biochem. Biophys. 230:1; King *et al.*, 1985, J. Allergy and Clin. Immunol. 75:621; King, 1987, J. Allergy Clin. Immunol. 79:113; Hoffman, 1985, J. Allergy and Clin. Immunol. 75:611). Their venom each contains three major venom allergens, phospholipase (37 kD), hyaluronidase (43 kD) and antigen 5 (23 kD) of as yet unknown biological function.

In addition to the insect venom allergens described above, the complete amino acid sequence of several major allergens from different grass (Perez *et al.*, 1990, J. Biol. Chem. 265:16210; Ansari *et al.*, 1989, Biochemistry 26:8665; Silvanovich *et al.*, 1991, J. Biol. Chem. 266:1204), tree pollen (Breiteneder, 1989, EMBO J. 8:1935; Valenta *et al.*, 1991, Science, 253:557), weed pollen (Rafnar *et al.*, 1991, J. Biol. Chem. 266:1229; Griffith *et al.*, 1991, Int. Arch. Allergy Appl. Immunol. 96:296), mites (Chua *et al.*, 1988, J. Exp. Med. 167:175), cat dander (Griffith *et al.*, 1992, Gene. 113:263), and mold (Aruda *et al.*, 1990, J. Exp. Med. 172:1529; Han *et al.*, 1991, J. Allergy Clin. Immunol. 87:327) have been reported. These major allergens are proteins of 10-40 kD and they have widely different biological functions. Nearly all allergens of known sequences have a varying extent of sequence similarity with other proteins in our environment. A comprehensive list of nearly all known allergens is maintained under the auspices of the World Health Organization (WHO) and International Union of Immunological Standards (IUIS) Sub-Committee for

Allergen Nomenclature, available at Internet site allergen.org on the World Wide Web.

T and B Cell Epitope of Allergens

Antibody responses to proteins require the collaboration of T helper and B lymphocytes and antigen presenting cells (APC). The antigen receptors of B cells are the membrane-bound antibody (Ab) molecules, which recognize and bind immunogens directly. The antigen receptors of T cells (TCR) only recognize and bind complexes of antigenic peptide-MHC class II molecule. Immunogens are first processed by APC into peptides that are presented on the surface of APC in association with the MHC class II molecules (Unanue, 1992, Current Opinion in Immunol 4:63). As MHC molecules are highly polymorphic in individuals, they have different specificity of binding antigenic peptides (Rothbard and Gefter, 1991, Ann. Rev. Immunol. 9:527). This is one mechanism for genetic control of immune response.

T helper cells are activated when the antigen receptor binds the peptide-MHC complex on the surface of APC. Activated T cells secrete lymphokines. In mice (Street and Mosmann, 1991, FASEB J. 5:171) and apparently in humans (Wierenga *et al.*, 1990, J. Immunol. 144:4651; Parronchi *et al.*, 1991, Proc. Natl. Acad. Sci. USA. 88:4538) the T helper cells can be divided into different types on the basis of their patterns of lymphokine production. Primarily, T helper cells divide into two groups: Th1 cells producing IL-2 and IFN-γ and Th2 cells producing IL-4 and IL-5. These lymphokines in turn influence the antigen-activated B cells to differentiate and proliferate into plasma cells secreting Abs of different isotypes. IL-4 is one lymphokine known to influence IgE synthesis (Finkelman *et al.*, 1990, Ann. Rev. Immunol. 8:303).

It is believed that the entire accessible surface of a protein molecule can be recognized as epitopes by the antigen receptors of B cells, although all epitopes are not necessarily recognized with equal likelihood (Benjamin *et al.*, 1984, Ann. Rev. Immunol. 2:67). B cell epitopes of a protein are of two types: topographic and linear. The topographic type consists of amino acid residues which are spatially adjacent but may or may not be sequentially adjacent. The linear type consists of only sequentially adjacent residues. X-ray crystallographic data of Ag-Ab complexes indicate the size of their complementary binding region to have 16-17 amino acid residues (Amit *et al.*, 1986, Science 233:747). Phospholipase, like other protein antigens, can have both types of B cell epitopes or only one.

Vespid antigen 5s have both types. Bee venom melittin appears to have only one B cell epitope of linear type (King *et al.*, 1984, J. Immunol. 133:2668).

T cell epitopes of proteins consist of only the linear type since they are peptides that have been processed in the lysosomes of APC by proteases (Unanue, 1992, Curr. Op. Immunol. 4:63). Analysis of naturally processed antigenic peptides bound to MHC class II molecules indicates that their size ranges from about 13 to 17 amino acid residues, but analysis of synthetic peptide-MHC class II molecule complex for their T cell proliferate response suggests a minimal size of about 8 amino acid residues (Cf. Rudensky *et al.*, 1991, Nature 353:622). Studies suggest that T cell epitopes are distributed throughout the entire protein molecule, and they may function as major or minor determinants depending on the MHC haplotype of the immunized host (Roy *et al.*, Science 244:572; Gammon *et al.*, 1987, Immunol. Rev. 98:53; O'Hehir *et al.*, 1991, Ann. Rev. Immunol. 9:67).

Hypersensitivity of the immediate type is known to be caused by the presence of allergen-specific IgE. IgE is found in the circulation and bound to specific IgE-Fc receptors on mast cells and basophils. Cross-linking of cell-bound IgE by allergens leads to release of histamine, leukotrienes and other chemical mediators that cause the allergic symptoms. IgE is one of the different isotypes of immunoglobulins. As pointed out above, lymphokines secreted by T cells influence isotype switch events in B cells.

Because of the central role of Th2 cells in determining the isotype switch event of B cells, the T cell epitopes of several allergens have been mapped (Cf. O'Hehir *et al.*, *supra*). These allergens include ragweed Amb III, rye grass Lol p I, cat Fel d I, mouse urine Mus m I, midge Chi t I, bee venom phospholipase A2 (Dhillon *et al.*, 1992, J. Allergy Clin. Immunol. 90:42) and melittin (Fehlner *et al.*, 1991, J. Immunol. 146:799). The data do not reveal any unusual or common structural features. However, any conclusion from these data is qualified as these data are collected from humans and mice of different haplotypes.

<u>Modulation of T and B Cell Responses</u>

Normally hosts are tolerant to the dominant B and T cell epitopes of self proteins by clonal deletion and anergy. However this tolerance can be broken under certain circumstances (Gammon *et al.*, 1991, Immunol. Today 12:193; Basten *et al.*, 1991, Immunol. Rev. 122:5). It has been suggested that self-tolerance is broken in autoimmune diseases through encounters with foreign proteins that are similar to host proteins. Therefore the

sequence similarity of allergens with autologous proteins is of interest for closer investigation.

Mature B cells are activated in response to multivalent antigens, which can cross-link cell surface Ig receptors (DeFranco, 1987, Ann. Rev. Cell Biol. 3:143), and they are rendered anergic in response to mono-valent antigen (Basten *et al.*, 1991, supra). Antigen activation of T cells requires not only the integration of TCR with peptide-MHC complex but also with other co-stimulating signals on the surface of APC (Schwartz, 1990, Science 248:1349; Jenkins and Miller, 1992, FASEB J. 6:2428). Interaction of TCR with peptide-MHC complex in absence of co-stimulating signals can lead to T cell anergy.

Experimental autoimmune encephalomyelitis (EAE) in mice or rats is a well-studied model for multiple sclerosis. Many studies have identified immunodominant T cell determinants for myelin basic protein, which is used to induce this condition. Peptides that correspond to immunodominant epitopes of myelin basic protein can induce tolerance to the same peptide antigen or to the intact myelin basic protein. The same peptides that induced tolerance could also induce T cell anergy in an ongoing autoimmune response (Gaur *et al.*, 1992, Science 259:1491-1494).

Early studies have shown that the physical state of the immunogen and the route of immunization are important variables in determining the outcome of an immune response. In the light of our current understanding, these variables may well influence antigen presentation so as to have T and B cell activation or anergy.

Immunotherapy

One way to treat allergic diseases is by immunotherapy, which involves repeated subcutaneous injections of the offending allergen(s) into patients. For most patients following immunotherapy, allergen-specific IgG levels initially rise. A gradual decrease of allergen-specific IgE levels follows the IgG rise (Norman, 1993, Current Op. Immunol. 5:968). Treated patients also show changes in their T cell cytokine profile: IL-4 and IL-5 levels decreased and IFN-γ level increased (Secrist *et al.*, 1993, J. Exp. Med. 178:2123.)

Studies have shown that immunotherapy with high doses of allergens is more effective for symptom reduction than that with low doses. However, effective dosages of allergens were limited by the potential danger of unwanted systemic allergic reaction in patients. Because of the undesirable systemic reaction on immunotherapy with native

allergens, there has been continued interest in the development of modified allergens with reduced allergenic activities for immunotherapy (T.P. King, 1993, in "Bronchial Asthma," edited by E.B. Weiss and M. Stein, Little Brown, Boston, pp. 43-49; R.E. O'Hehir *et al.*, 1991, *supra*).

Allergenicity depends on the interaction of a multi-valent allergen with basophil or mast cell-bound IgE antibodies. Therefore, allergenicity of a protein can be reduced by decreasing its B cell epitope density. Reduction of B cell epitope density of a protein can be accomplished by several approaches. One approach is by partial or complete denaturation of allergens by chemical treatment or fragmentation (Takatsu *et al.*, 1975, J Immunol 115:1469; Pesce *et al.*, 1990, Int Arch Allergy Appl Immunol 92:88; Vrtala *et al.*, 1997, J Clin Invest 99:1673) since the majority of B cell epitopes are of the discontinuous type, i.e., dependent on the native conformation of proteins. For example, urea treatment of the major allergen from ragweed pollen led to irreversible denaturation with loss of the discontinuous B cell epitopes but retention of the continuous B and T cell epitopes (Takatsu *et al.*, 1975, J Immunol 115:1469). Immunotherapy of patients with the fully denatured ragweed allergen showed no changes in specific IgE and IgG levels for the native allergen although the peripheral blood mononuclear cells of treated patients did show decreased proliferative response on antigen stimulation (Norman *et al.*, 1980, J Allergy Clin Immunol 66:336). Use of partially denatured allergens has also been proposed. This is exemplified by the recombinant mite allergens, which lack the cysteine residues that are involved in maintaining the native structure of the protein (Smith *et al.*, 1996, Mol Immunol 33:399; T. Takai *et al.*, 1997, Nature Biothechnology 15:754).

Two reports have appeared on the use of T cell epitope peptides to modulate allergen-specific immune responses. One report is on the subcutaneous injection of mice with two peptides from the major cat allergen Fel d I to decrease T cell response to the entire molecule Fel d I (Briner *et al.*, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:7608-12). Another is on the intranasal therapy with a peptide from the major mite allergen Der p I to suppress allergen-specific response in naive or sensitized mice (Hoyne *et al.*, 1993, J. Exp. Med. 178:1783-1788).

These findings suggested the use of T cell peptides as immunotherapeutic reagents since T cell peptides are like the denatured allergens in that they lack the

discontinuous B cell epitopes. The dominant T cell peptides of several allergens were tested in patients; cytokine level changes but not antibody level changes were observed (Muller *et al.*, 1998, J Allergy Clin Immunol 101:747; Simons *et al.*, 1996, Int Immunol 8:1937; Creticos *et al.*, 1997, J Allergy Clin Immunol 99:401; Marcotte *et al.*, 1997, J Allergy Clin Immunol 99:405). Importantly, these clinical findings with the urea-denatured allergen and T cell peptides suggest that the retention of the discontinuous B cell epitopes as well as the continuous B and T cell epitopes is required for modified allergens to be effective in modulating both antibody and cellular immune responses.

A second approach to reduce the accessibility of B cell epitopes of allergen involves polymerization of the allergen by formaldehyde or glutaraldehyde treatment (Marsh, 1971, Int Arch Allergy Appl Immunol 41:199; Patterson *et al.*, 1973, J Immunol 110:1413) or by attachment of non-immunogenic polymers (King *et al.*, 1979, J Exp Med 149:424). Glutaraldehyde polymerized antigens were found to be processed differently from the natural antigens in mice, and they were processed by antigen-presenting cells that secrete cytokines promoting Th1 responses (Gieni *et al.*, 1993,. J Immunol 150:302). This second approach for improved immunotherapy had been tried with ragweed pollen allergens with immunological findings similar to those with natural allergens (Norman *et al.*, 1982, J Allergy Clin Immunol 70:248; Norman, 1984, J Allergy Clin Immunol 73:787). One limitation of this approach was that near complete loss of the discontinuous B cell epitopes usually occurred when allergens were modified to achieve greater than100-fold reduction in allergenicity.

A third approach is by site-directed mutagenesis to selectively alter the contact amino acid residues of B cell epitopes of allergens. If the key contact residues of B cell epitopes are known, this can be a useful approach. For example, a single residue mutation of Glu to Ser in the major birch allergen abolished its binding of a murine antibody, and resulted in a 40% decrease of its binding of IgEs from a serum pool of allergic patients (Mirza *et al.*, 2000, J Immunol. 165:331). The different decreases probably reflect that the murine antibody and the human IgEs are respectively of monoclonal and polyclonal origins.

Since an MHC class II molecule of any one haplotype can bind a wide range of peptides in its binding groove, it may be possible to modulate T cell response by inhibition of allergen-derived T cell epitope binding to MHC molecules with other peptides. For

example, a mouse lysozyme peptide which is not immunogenic by itself in H-2k mice inhibits T cell response to hen egg white lysozyme (Adorini and Nagy, 1990, Immunol. Today 11:21). Another example is the *in vitro* inhibition of T cell response to a mite allergen by an influenza HA peptide (O'Hehir *et al.*, 1991, J. Allergy Clin. Immunol. 87:1120).

Immune response to an immunogen/allergen thus depends in part on the genetic make-up of the host, the route and mode of immunization and the immunogen/allergen. The extent to which an allergen determines the outcome of IgE response is not known. How many B and T cell epitopes must each allergen have? Are immunodominant B or T cell epitopes of an allergen recognized by different or all susceptible individuals? Are there T cell epitopes which favor IgE class switch events in B cells? Does antigenic cross reactivity of allergens with host proteins play a role as to why some proteins are more allergenic than others are? Can tolerance to a multi-valent allergen be induced by treatment with a single or a combination of B or T cell epitopes?

U.S. Patent Nos. 5,593,877; 5,612,209, 5,804,201, 6,106,844, 6,270,763 and 6,287,559 and U.S. application Serial No. 09/166,205 to King disclose the isolation of cDNAs encoding vespid venom proteins and the deduced amino acid sequences of proteins encoded by the cDNAs. The cDNAs allow the expression and purification of large quantities of vespid venom proteins and polypeptides for use in immunotherapy. Sequences, however, fail to yield information on the native structure of vespid venom. Hence, the cDNAs and deduced amino acid sequences do not yield information on discontinuous epitopes. Nor do the deduced vespid venom amino acid sequences predict epitopes that will be present on the surface of recombinantly produced vespid venom proteins. Consequently, the cDNA and deduced amino acid sequences alone cannot accurately predict which regions or peptides of vespid venom proteins will serve as efficient immunogens to stimulate a B cell-mediated immune response. Nor can the cDNA and deduced amino acid sequences alone predict the epitope density on the surface of a vespid venom protein, which is an important determinant of the potential to crosslink surface IgE molecules, and hence the allergenicity, of a vespid venom protein.

Thus, there is a need in the art to determine how modification of B cell epitopes in the native structure of allergen proteins permits the design of improved therapeutics.

There is also a need in the art to provide allergen proteins that stimulate a B cell-mediated immune response without stimulating IgE mediated allergic responses. In particular, there is need in the art for providing allergens with a reduced density of epitopes that are efficient in stimulating an IgG production in B cells but are inefficient at crosslinking IgE antibodies specific for the native allergen bound to the surface of, for example and without limitation, mast cells or basophils.

There is also a need in the art to provide hybrid proteins bearing non-cross-reactive B cell epitopes that are effective in immunotherapy. In particular there is a need to for hybrid proteins that present allergen peptide epitope sequences in a conformation that is accessible to receptors on the surface of immune cells and soluble proteins, especially antibodies.

Hence, what are needed are agents, pharmaceutical compositions and methods for generating an IgG B cell response that provides protection against allergens, without eliciting an allergic reaction such as anaphylactic shock.

The citation of references herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY OF THE INVENTION

The present invention provides a new approach to prepare modified allergens. The modified allergens are hybrids consisting of a small portion of the "guest" allergen of interest and a large portion of a homologous but poorly cross-reacting "host" protein. The homologous host protein functions as a scaffold to maintain the native structure of the guest allergen of interest so that the conformation-dependent B cell epitopes of the guest allergen of interest are preserved in the hybrid, but at a reduced density. Homologous proteins of greater than 30% sequence identity and of similar functions are known to have closely similar three-dimensional structures (Chothia *et al.*, 1990, Annual Review Biochem 59:1007; Russell, 1994, J Mol Biol 244:332), thus providing a plethora of guest/host proteins.

Thus, the present invention is directed to recombinant allergens, e.g., vespid venom allergens, of reduced allergenicity but that retain immunogenicity. Hence, the invention provides allergen protein, peptide epitope sequences corresponding to surface-accessible portions of the allergen, hybrid proteins comprising the peptide epitope sequences

inserted in the corresponding structural region of the host scaffold, nucleic acids encoding such hybrid constructs, and methods that may be used to stimulate a therapeutic immune response to the allergens with reduced allergic response, i.e., an allergy immunotherapy. In particular, the recombinant hybrid proteins, nucleic acids and methods of the invention provide for stimulating a B cell-based response against the allergen, without triggering an IgE-based allergic response such as acute anaphylaxis.

The hybrid proteins of the present invention are present in a native conformation. In one embodiment hybrid proteins comprise at least one allergen peptide epitope sequence in a native conformation. More specifically, the scaffold protein and the native protein from which the allergen peptide epitope sequence is derived have the same native conformation.

In certain embodiments the hybrid proteins of the invention comprise a fusion peptide, such as a signal peptide or handle for purification. In other embodiments the hybrid proteins of the invention may comprise a protease processing site, e.g., for cleavage of the purification handle. Accordingly, the hybrid proteins of the invention comprises an allergen peptide epitope sequence, a scaffold protein sequence, and, optionally, either separately or in combination, a fused sequence and protease processing site.

The recombinant peptide epitope sequences are found on the surface of the native protein from which the sequence is derived. In a specific embodiment, the allergen peptide is a loop region of the native protein.

It will be appreciated that hybrid proteins may comprise more than one peptide epitope sequence introduced into the scaffold protein sequence.

The present invention extends to hybrid proteins wherein the peptide antigen is from a allergen protein and the scaffold protein is a heterologous protein having greater than or equal to 30% sequence identity to the native allergen protein. In a specific aspect, each of the peptide antigen and the scaffold protein are derived from vespid venom proteins. More specifically, the peptide antigen and scaffold proteins may be derived from vespid venom Ag 5s.

In one embodiment, the peptide epitope sequences of the present invention are characterized by having between about 6 and 50 amino acids and being antigenic in a mouse for a B cell response (B cell epitopes). More particularly, in examples of the invention, an

allergen peptide epitope sequence of the invention is derived from an Ag 5 peptide selected from the group consisting of:

NNYCKIKC (SEQ ID: 1);
NNYCKIKCLKGGVHTACK (SEQ ID: 2);
NNYCKIKCLKGGVHTACKYGSLKP (SEQ ID: 3);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVV (SEQ ID: 4);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQ (SEQ ID: 5);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILK (SEQ ID: 6);
QVGQNVALTGSTAAKYDDPVKLVKMWEDEVKDYNPKKKFSGNDFLKTG (SEQ ID NO: 7);
HYTQMVWANTKEVGCGSIKYIQEKWHKHYLVCNYGPSGNFKNEELYQTK (SEQ ID NO: 8)
LKPNCGNKVVV (SEQ ID NO: 9);
LTGSTAAKYDD (SEQ ID NO: 10);
PKKKFSGND (SEQ ID NO: 11)
IQEKWHK (SEQ ID NO: 12); and
FKNEELYQTK (SEQ ID NO: 13);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILKEHND (SEQ ID NO: 93);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILKEHNDFRQKIAR (SEQ ID NO: 94);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILKEHNDFRQKIARGLETRGNPGPQPPAKNMKN (SEQ ID NO: 95).

The present invention further extends to an isolated expression vector comprising a promoter operationally associated with a nucleic acid of the invention. Numerous promoters commercially available to the skilled artisan can be used in this aspect of the invention. Examples include, but are not limited to immediate early promoters of hCMV, early promoters of SV40, early promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late promoters of SV40, late promoters of adenovirus, late

promoters of vaccinia, late promoters of polyoma, the lac the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage lambda, control regions of fd coat protein, 3-phosphoglycerate kinase promoter, acid phosphatase promoter, or promoters of yeast α mating factor, to name only a few. Numerous examples of expression vectors having applications herein, and which are also readily available to the skilled artisan are described infra.

The invention also provides a method for preparing a nucleic acid that encodes an allergen hybrid protein of the invention. This method comprises introducing a nucleotide sequence encoding a peptide epitope sequence of an allergen protein into a nucleotide sequence encoding a scaffold protein that is structurally homologous to the allergen protein. The nucleotide sequence encoding the peptide epitope sequence is introduced in-frame with the nucleotide sequence encoding the scaffold protein, and in a location such that in the allergen hybrid protein the peptide epitope sequence is present in a surface accessible region of the hybrid protein corresponding to its position in the allergen protein. In one such embodiment, the nucleotide sequence encoding the scaffold protein is mutated to introduce the nucleotide sequence encoding the peptide epitope sequence. In another such embodiment, the nucleotide encoding the peptide epitope sequence is introduced by ligating fragments from nucleic acids comprising the nucleotide sequence encoding the peptide epitope sequence and the nucleotide sequence encoding the scaffold protein treated with an endonuclease. If necessary, endonuclease restriction sites can be introduced into the nucleic acids comprising such sequences using standard techniques in the art.

The present invention further extends to a method for producing a hybrid protein of the invention by expression of an isolated nucleic acid molecule of the invention. Such production provides a plentiful source of the hybrid protein for diagnosis and therapy. An example of such a method of the invention for producing a hybrid protein culturing a host cell transformed or transfected with an expression vector of the invention so that the host cell produces the hybrid protein of the invention. Preferably, the hybrid protein of the invention so produced from the culture, the host cell, or both is recovered.

The present invention further extends to pharmaceutical compositions effective for the treatment of an allergen-specific allergic condition. In particular, the present

invention extends to a pharmaceutical composition comprising a hybrid protein of the invention, or a nucleic acid preferably an expression vector, encoding such a hybrid protein, and a pharmaceutically acceptable carrier thereof. The invention further includes pharmaceutical compositions containing a plurality of hybrid proteins of the invention, or containing a nucleic acid or nucleic acids encoding such a plurality.

Naturally, the present invention extends to a method for treating allergen-specific allergic condition comprising administering a therapeutically effective amount of a pharmaceutical composition of the invention. Administration of a pharmaceutical composition of the invention can occur by any route, and particularly orally, pulmonarily, nasally, topically or parenterally. Other routes of administration are also possible.

Yet another specific object of the invention is to provide a method for treating an allergen-specific allergy in a subject, wherein a pharmaceutical composition for treating an allergen-specific allergic condition is administered to the subject.

Moreover, the present invention extends to a pharmaceutical composition for modulating immune response of a mammal towards an immunogen, wherein the pharmaceutical composition comprises an allergen hybrid protein (or nucleic acid encoding such a protein) of the invention for modulating immune response towards an immunogen in a mammal, as set forth above, and a pharmaceutically acceptable carrier thereof.

As a result, administration of such a pharmaceutical composition modulates the immune system's ability to recognize and attack the immunogen. In a particular embodiment, the ability of the immune system of the mammal to recognize and attack the immunogen is increased upon administration of the pharmaceutical composition relative to the ability of the subject's immune system to recognize and attack the immunogen prior to administration of a pharmaceutical composition of the invention.

ABBREVIATIONS

Dol m	Dolichovespula maculata	white faced hornet
Dol a	D. arenaria	yellow hornet
Pol a	Polistes annularis	wasp
Pol e	P. exclamans	wasp
Ves m	Vespula maculifrons	yellowjacket

Ves v	V. vulgaris	yellowjacket
PCR		polymerase chain reaction
RACE		rapid amplification of cDNA ends
TCR		T cell receptor for antigen

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Ves v 5 cDNA [SEQ ID NO: 14] and amino acid [SEQ ID NO: 16] sequences. Numbering at L refers to nucleotide position; numbering at R refers to amino acid position.

Fig. 2. Pol a 5 cDNA [SEQ ID NO: 15] and amino acid [SEQ ID NO: 17]sequence. Numbering at L refers to nucleotide position; numbering at R refers to amino acid position.

Fig. 3. Amino acid comparison of Ves v 5 (V) [SEQ ID NO: 16] and Pol a 5 (P) [SEQ ID NO: 17].

Fig. 4. Schematic sequence representations of Ag 5s and hybrids. Residue numbers given for hybrids refer to those of Ves v 5.

Fig. 5A-B. Alignment of Ves v 5 homologous proteins from insect venoms from Vespula maculifrons [Ves m 5, SEQ ID NO: 63]; Vespula vulgaris [Ves v 5, SEQ ID NO: 64]; Vespula flavopilosa [Ves f 5, SEQ ID NO: 65]; Vespula pensylvanica [Ves p 5, SEQ ID NO: 66]; Vespula germanica [Ves g 5, SEQ ID NO: 67]; Vespula vidua [Ves vi 5, SEQ ID NO: 68]; Vespula squamosa [Ves s 5, SEQ ID NO: 69]; Dolichovespula maculata [Dol m 5a, SEQ ID NO: 70]; Dolichovespula arenaria [Dol a 5, SEQ ID NO: 71]; Dolichovespula maculata [Dol m 5b, SEQ ID NO: 72]; Vespa mandarinia [Vesp m 5, SEQ ID NO: 73]; Vespa crabro [Ves c 5.01, SEQ ID NO: 74]; Vespa crabro [Ves c 5.02, SEQ ID NO: 75]; Polistes fuscatus [Pol f 5, SEQ ID NO: 76]; Polistes exclamans [Pol e 5, SEQ ID NO: 77]; Polistes annularis [Pol a 5, SEQ ID NO: 78]; Solenopsis invicta [Sol i 3, SEQ ID NO: 79]; and Solenopsis richteri [Sol r 3, SEQ ID NO: 80].

Fig. 6A-B. SDS gel patterns of Ag 5s and hybrids.

Fig. 7. Circular dichroism (CD) spectra of Ves v 5 and hybrids.

Fig. 8A-C. Inhibition ELISA with mouse antibodies specific for natural Ves v 5 using (A) Ves v 5-specific antibodies isolated from BALB/c mice and depleted of Pol a-cross reactive antibodies (B) antisera from ASW/n mice and (C) antisera from P/J mice.

Fig. 9A-C. Inhibition ELISA with sera from yellow jacket-sensitive patients.

Fig. 10 A-C. Binding of mouse Ves v 5-specific monoclonal antibodies to solid-phase Ves v 5 or hybrids.

Fig. 11 A-C. Histamine release assay of Ves v 5, Pol a 5 and hybrids.

Fig. 12A-B. Alignment of Ves v 5-like proteins. Aligned proteins are Ves v 5 [SEQ ID NO: 81]; Sol i 3 [SEQ ID NO: 82]; Lycopersicon esculentum p14a [SEQ ID NO: 83]; Schizophyllum commune SC7 [SEQ ID NO: 84]; human trypsin inhibitor [SEQ ID NO: 85]; human glipr [SEQ ID NO: 86]; Heloderma horridum helothermine [SEQ ID NO: 87]; and human TPX-1 [SEQ ID NO: 88].

DETAILED DESCRIPTION

The present invention is directed to recombinant allergen hybrid protein constructs of reduced allergenicity and but retaining immunogenicity, the nucleic acid molecules encoding such allergens, and methods of use for such allergens in the diagnosis and therapy of allergy. The hybrid proteins of the invention comprise a surface, e.g., loop or corner region, peptide epitope sequence introduced into a scaffold protein sequence. The hybrid proteins, nucleic acids and methods of the invention provide for stimulating a B cell-based response against the allergen without triggering an IgE-based allergic response. In a specific embodiment, a recombinant hybrid protein comprises a vespid venom surface or loop peptide antigen, particularly from Ves v 5, fused to a scaffold protein, particularly Pol a 5.

The invention is further directed to expression vectors comprising nucleic acid molecules that include allergen hybrid proteins of decreased allergenicity that retain immunogenicity, and to methods for producing such hybrid proteins of the invention by expressing and recovering such hybrid proteins.

The invention also provides pharmaceutical compositions effective for the treatment of an allergen-specific allergic condition comprising a hybrid protein of the invention or nucleic acid vector encoding such a hybrid protein, and methods for treating

such allergic conditions comprising administering a therapeutically effective amount of such pharmaceutical compositions.

The hybrid proteins of the invention can also be useful for diagnosis of allergen-specific allergic conditions.

The present invention is based, in part, on the discovery that insertion of sequences from surface accessible regions of yellowjacket (*Vespula vulgaris*) antigen 5 into the corresponding region of *Polistes annularis* antigen 5 yielded a hybrid construct that retained the immunogenicity of the parent proteins, but showed significantly reduced allergenicity. Moreover, the most advantageous positions for introducing sequences were at surface accessible sites, especially loop and corner regions, as determined from the crystal structure of Ves v 5.

Earlier work established that hybrid constructs, in which one-quarter to one-third of the allergenic protein was introduced into the corresponding region of a homologous scaffold protein. However, these hybrid constructs lack the advantages and refinements of the present invention.

Clinical studies in patients and tests with experimental animals have shown that there is limited cross reactivity of antibodies specific for the yellow jacket and paper wasp venom proteins (Lichtenstein *et al.*, 1979, J Allergy Clin Immunol 64:5; Lu *et al.*, 1993, J Immunol 150:2823). These observations form the basis of a preferred embodiment of the present invention. A preferred guest allergen antigen 5 is Ves v 5, a yellow jacket venom protein of 23 kd. A preferred homologous host allergen, which serves as a scaffold protein, is Pol a 5, a paper wasp venom protein of similar size. Ves v 5 and Pol a 5 have 59% sequence identity (Fig. 3). Both can be expressed in yeast and the recombinant proteins were shown to have the native conformation of the natural proteins (Monsalve *et al.*, 1999, Protein Expr. Purif. 16:410).

Immunochemical findings are reported for hybrids of Ves v 5 and Pol a 5. The sequence representations of these hybrids are shown schematically in Fig. 4. Hybrids PV1-46, PV109-155 and PV156-204 contain respectively the first one-quarter (i.e., amino acids 1-46), the third one-quarter (i.e., amino acids 109-155) and the last one-quarter (i.e., amino acids 156-204) of the Ves v 5 molecule, together with portions of the Pol a 5 molecule to complete the hybrid Ag 5 molecule. A hybrid containing the second one-quarter of the

Ves v 5 molecule was not prepared, as this is a region of high sequence identity of Ves v 5 and Pol a 5 (see Fig. 3). Hybrid PV1-155 has the opposite arrangement of the Ves v 5 and Pol a 5 amino-terminal and carboxy-terminal fragments, when compared to PV156-204.

Hybrids PV1-8, PV1-18, PV1-24, PV1-32, PV22-32, PV115-125, PV142-150, PV176-182 and PV195-204 were designed to contain the surface, loop or corner regions of Ves v 5. These hybrids include 7-32 amino acids of Ves v Ag 5 substituted for a homologous region of Pol a Ag 5.

Switching corresponding regions of homologous proteins, especially in surface accessible, e.g., loop and corner, regions predictably conserves native structure. Surface accessible regions especially loop and corner regions, tend to demonstrate more flexibility and better tolerate changes while retaining structure. This approach also finds a counterpart in directed evolution, where homologous enzymes are recombined to yield novel, functional enzyme chimeras.

The term "allergen hybrid protein" refers to a recombinant or synthetic protein that has the native structure of the scaffold protein, but includes one or more sequences from an allergen. The allergen is a structural homolog of the scaffold protein, thus permitting introduction of the allergen sequences into corresponding positions in the scaffold protein. A "corresponding position" is the same position in the primary sequence or same topological position in the native structure. The allergen sequences are selected from a surface accessible region of the allergen and inserted in the corresponding surface accessible region of the scaffold protein. Because B cell epitopes of proteins in their native conformation are surface accessible, the sequences from the allergen introduced into the scaffold protein can act as B cell epitopes, hence they are called "peptide epitope sequences" of an allergen protein.

In connection with the present invention the expression "reduced allergenicity" means a molecule or antigen exhibits significantly reduced allergenic activity in an in vitro assay designed to measure such allergenicity. Such *in vitro* assays are well known in the art and include, for example and without limitation, assay of histamine release from basophils of a allergen sensitive patient or experimental animal following challenge. Furthermore, "activity" as used herein may refer to any measurable parameter or result that is indicative of the allergenicity of a molecule or antigen, such as, for example and without

limitation, the maximum response obtained in an assay or the amount or concentration of antigen required to elicit a defined result in an assay.

The term "retaining immunogenicity" (in any grammatical form) means that the hybrid protein elicits an immune response, particularly an IgG-predominated humoral immune response, that is comparable to the immune response elicited by the native allergen or scaffold protein (or both) and greater than the allergic (IgE) immune response they elicit. The hybrid-specific IgG will cross react with epitopes present on the allergen and the scaffold protein. This IgG response can block IgE binding, thus reducing or preventing allergic responses. In addition, the hybrid protein may elicit T cell anergy and other allergy suppressive immune responses.

In accordance with the present invention, proteins are "homologous" if, following alignment, they exhibit at least about 30 percent amino acid identity, as determined by programs that are well know in the art, including, as non-limiting examples, the programs Gap, Bestfit and BLAST. More preferable is where homologous proteins exhibit at least 50 percent amino acid identity. However, in a specific embodiment the allergen protein and the scaffold protein do not have more than 70% sequence identity to reduce the possibility of a high degree of cross reactivity that might lead to an unaccepatable degree of allergenicity of the hybrid protein. Greater sequence identity can be tolerated, particularly where the peptide epitope sequence inserted in the scaffold protein is very dissimilar, e.g., less than 50% identical and preferably less than 30% identical, to the corresponding sequence from the scaffold protein that it replaces.

Proteins are structurally homologous when, due to primary sequence similarity, they adopt a similar core secondary and tertiary structure so that their three-dimensional structures can be superimposed with almost complete (greater than 70%) overlap. Their surface tertiary structure, however, may vary.

In a preferred embodiment of the present invention, peptide epitope sequences from the allergen are inserted into or replace sequences within "scaffold" proteins. Accordingly, a "scaffold protein" of the present invention is a protein which includes an allergen epitope sequence, either as an inserted sequence or as a replacement sequence for a homologous (corresponding) sequence of the scaffold protein. The scaffold protein adopts a native conformation. The allergen and scaffold can alternate positions; these terms are used

to indicate the source of sequences (from the "allergen") introduced into the "scaffold". Because the "allergen" and "scaffold" are homologous, they are both likely to act as allergens, albeit to different populations. Thus, a "scaffold" can be an "allergen" if its surface accessible sequences are introduced into another structurally homologous protein.

The expression "native conformation" includes a functional conformation adopted by a non-recombinant, *i.e.*, natural protein, polypeptide, or antigen, within its natural environment or following purification under conditions that maintain the functional conformation adopted in said natural environment. Native conformation can be measured, for example and without limitation, by determining the CD spectrum of a protein. Native conformation may also be determined by measuring enzymatic activity. It will be understood by the skilled artisan that, in cases where the functional conformation of a natural non-recombinant protein is unknown, "native conformation" will encompass forms of recombinant proteins that reproducibly exhibit a non-random defined conformation that includes secondary elements as typically found in properly folded functional proteins, such as for example, and without limitation, α helix and β sheet elements. It is also well known that, using recombinant techniques, additional amino acids may be joined to the amino or carboxyl end of a protein without disrupting the native conformation of the protein. Such additional amino acids may be short polypeptide "tags", which are typically 1-25 amino acids in length and which are typically disordered, or longer polypeptides which may form a distinct domain, which may itself be ordered or disordered.

The expression "surface-exposed amino acid" means that an amino acid residue is located at the surface of the three-dimensional structure in such a manner that when the allergen is in solution at least a part of at least one atom of the amino acid residue is accessible for contact with the surrounding solvent. Preferably, the amino acid residue in the three-dimensional structure has a solvent (water) accessibility of at least 20%, more preferably at least 30 %, still more preferably at least 40 % and most preferably at least 50%.

The expression "solvent accessibility" is defined as the area of the molecule accessible to a sphere with a radius comparable to a solvent (water, r = 1.4 Å) molecule.

An "allergen" has its ordinary meaning, i.e., is any proteinacious molecule that elicits an allergic response, e.g., histamine release to anaphylactic shock. Allergens are well known; a representative group are listed in Table 8 of this specification. Examples of allergens according

to the invention may suitably be an inhalation allergen originating, e.g., from trees, grasses, herbs, fungi, house dust mites, cockroaches and animal hair and dandruff. Important pollen allergens from trees, grasses and herbs are such originating from the taxonomic orders of *Fagales*, *Oleales* and *Pinales* including birch (*Betula*), alder (*Alnus*), hazel (*Corylus*), hornbeam (*Carpinus*) and olive (*Olea*), the order of *Poales* including i.a. grasses of the genera *Lolium*, *Phleum*, *Poa*, *Cynodon*, *Dactylis* and *Secale*, the orders of *Asterales* and *Urticales* including herbs of the genera *Ambrosia* and *Artemisia*. Important inhalation allergens from fungi are such originating from the genera *Alternaria* and *Cladosporium*. Other important inhalation allergens are those from house dust mites of the genus *Dermatophagoides*, those from cockroaches and those from mammals such as cat, dog and horse. Further, recombinant allergens according to the invention may be mutants of venom allergens including such originating from stinging or biting insects such as those from the taxonomic order of *Hymenoptera* including bees (superfamily Apidae), wasps (superfamily Vespidea), and ants (superfamily Formicoidae). Specific allergen components include, e.g., *Bet v* 1 (*B. verrucosa*, birch), *Aln g* 1 (*Alnus glutinosa*, alder), *Cor a* 1 (*Corylus avelana*, hazel) and *Car b* 1 (*Carpinus betulus*, hornbeam) of the *Fagales* order. Others are *Cry j* 1 (*Pinales*), *Amb a* 1 and 2, *Art v* 1 (*Asterales*), *Par j* 1 (*Urticales*), *Ole e* 1 (*Oleales*), *Ave e* 1, *Cyn d* 1, *Dac g* 1, *Fes p* 1, *Hol l* 1, *Lol p* 1 and 5, *Pas n* 1, *Phl p* 1 and 5, *Poa p* 1, 2 and 5, *Sec c* 1 and 5, and *Sor h* 1 (various grass pollens), *Alt* a 1 and *Cla h* 1 (fungi), *Der f* 1 and 2, *Der p* 1 and 2 (house dust mites, *D. farinae* and *D. pteronyssinus*, respectively), *Lep d* 1 and 2 (Lepidoglyphus destructor; storage mite), *Bla g* 1 and 2, *Per a* 1 (cockroaches, *Blatella germanica* and *Periplaneta americana*, respectively), *Fel d* 1 (cat), *Can f* 1 (dog), *Equ c* 1, 2 and 3 (horse), *Apis m* 1 and 2 (honeybee), *Ves v* 1, 2 and 5, *Pol a* 1, 2 and 5 (all wasps) and *Sol i* 1, 2, 3 and 4 (fire ant). The term also includes all examples described in the "Background", *supra*.

For example, the term "vespid venom allergen" refers to a protein found in the venom of a vespid, to which susceptible people are sensitized on exposure to the sting of the insect. While most antigens are characterized by being reactive with specific IgG class antibodies, an allergen is characterized by also being reactive with IgE type antibodies. The IgE type antibodies are responsible for mediating the symptoms of an allergic condition, *i.e.*, immediate-type hypersensitivity.

As used herein, the term "vespid" is used according to the practice of those in the field of allergy, and refers to insects belonging to the worldwide family of Vespidae, *i.e.*, social wasps including hornets, yellowjackets, and paper wasps. In particular, vespids include the subfamilies Vespinae and Polistinae. More particularly, the vespids include the

genera Vespa Linnaeus, Vespula Thomson, Dolichovespula Rohwer, and Polistes Latreille. Species in the genus Vespula include but are not limited to V. germanica (Fab.), V. squamosa (Drury), V. maculifrons (Buysson), V. flavopilosa (Jacobson), V. vulgaris (L.), and V. pensylvanica (Saussure). Species in the genus Polistes include but are not limited to P. annularis (Linnaeus), P. exclamans (Viereck), P. metricus (Say), P. fuscatus (Fabricius), and P. apachus (Saussure). Species in the genus Dolichovespula include but are not limited to D. maculata (L.) and D. arenaria (Fab.). Species in the genus Vespa include but are not limited to V. crabro (L.) and V. orientalis (Linnaeus).

The taxonomic classification of Vespula vulgaris is as follows:

Order:	Hymenoptera
Suborder:	Apocrita
Division:	Aculeata
Superfamily:	Vespoidea
Family:	Vespidae
Subfamily:	Vespinae
Genus:	Vespula
Species Group:	Vespula vulgaris species group
Species:	vulgaris

The taxonomic classification for Polistes annularis is as follows:

Order:	Hymenoptera
Suborder:	Apocrita
Division:	Aculeata
Superfamily:	Vespoidea
Family:	Vespidae
Subfamily:	Polistinae
Tribe:	Polistini
Genus:	Polistes
Subgenus:	Aphanilopterus
Species:	annularis

As used herein, the term "immunomodulatory" refers to an ability to increase or decrease an antigen-specific immune response, either at the B cell or T cell level. Immunomodulatory activity can be detected, *e.g.*, in T cell proliferation assays, by measurement of antibody production, lymphokine production or T cell responsiveness. In particular, in addition to affects on B cell responses, the immunomodulatory polypeptides of

the invention may bind to molecules on the surface of T cells, and affect T cell responses as well.

As used herein, the phrase "immune system related disease or disorder" refers to a disease or disorder that evokes an immune response in a subject, or effects the ability of the immune system to respond to an immunogen. Hence, examples of immune system related diseases or disorders comprise a pathogenic disease or disorder; a viral disease or disorder, *e.g.*, HIV, Herpes Simplex virus, or papilloma virus; an autoimmune disease, *e.g.*, arthritis or Lupus.

Determining Allergen Structure

The three-dimensional structure of a protein may be determined by physical methods that are well known in the art, including and without limitation, x-ray crystallography, nmr spectroscopy and electron crystallography. Preferred, the three-dimensional structure of a protein is determined by x-ray crystallography. It is also preferred that such techniques yield a resolution of 5Å or better, at which resolution a trace of the α-carbons in the polypeptide backbone of a protein may be obtained, allowing the determination of protein secondary structure features, as for example, α-helix and β-sheet elements. More preferred is where the three dimensional structure of protein is determined at a resolution of 2Å or better, at which resolution the position of amino acid side chains may be ascertained. Structures of specific allergens are well known, as set forth in Table 9. These, or others, can be determined using the standard techniques set forth above.

The three dimensional structure of a protein may also be inferred by comparison to an homologous protein, whose structure has been determined empirically by a physical method, as for example by aligning and comparing amino acid sequences. Methods for comparing and aligning amino acid sequences are well known in the art and include, for example and without limitation, the Pileup, Gap, BestFit and Compare programs (Genetic Computer Group, Madison, WI). Such alignment and comparison allows the identification of regions of high amino acid identity or similarity, which may adopt similar or identical conformations in homologous proteins. In this manner, once the three dimensional structure is determined for one protein, the three-dimensional structure may be determined for many homologous proteins, which allows for the identification of surface and loop regions of homologous proteins.

The three dimensional structure and function of a proteins is typically effected to a lesser extent by changes in amino acids located in surface and loop regions of proteins, compared to effects observed due to changes in internally located amino acids. The amino acid residues of surface and loop regions are therefore typically less conserved among homologous proteins, compared to internal residues. It will be appreciated by one of ordinary skill in the art, however, that surface and loop regions will occupy the same relative position in the native conformation of homologous proteins. The surface and loop regions therefore represent "conserved elements" or "homologous elements" within homologous proteins.

In addition, various spectroscopic techniques can be used to evaluate structure, particularly to confirm that the hybrid protein retains the native structure of the allergen and scaffold proteins. These techniques include, without limitation, circular dichroism spectroscopy, nmr spectroscopy (particularly at lower resolution), neutron diffraction, fluorescence spectroscopy (and other light absorption and transmission spectroscopic techniques), and the like. In particularly, evaluating identity of spectra can indicate the degree to which the hybrid protein adopts the native conformation. Circular dichroism spectroscopy provides a preferred tool for this type of evaluation.

Molecular Biological Techniques

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, "Molecular Cloning: a Laboratory Manual," Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook *et al.*, 1989"); "DNA Cloning: a Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J.Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984). Other techniques in accordance with the present invention may be found in U.S. Patent Nos. 5,593,877; 5,612,209, 5,804,201, 6,106,844 and U.S. application Serial Nos.

08/484,388, 08/474,853, and 09/166,205 to King and in Monsalve *et al.* (1999, Protein Expr. Purif. 16:410).

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear or circular DNA molecules, restriction fragments, viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook *et al.*, supra). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acid molecules, low stringency hybridization conditions, corresponding to a Tm of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% non-fat dry milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher Tm, *e.g.*, 40% formamide, with 5x or 6x SSC. High stringency hybridization conditions correspond to the highest Tm, *e.g.*, 50% formamide, 5x or 6x SSC. Hybridization requires that the two nucleic acid molecules contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acid molecules depends on the length of the nucleic acid molecules and the degree of complementation, variables well known in the art. The greater the degree of

similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acid molecules having those sequences. The relative stability (corresponding to higher Tm) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook *et al.*, supra, 9.50-0.51). For hybridization with shorter nucleic acid molecules, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook *et al.*, supra, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid molecule is at least about 10 nucleotide; more preferably the length is at least about 20 nucleotides; even more preferably at least about 30 nucleotides; and most preferably at least about 40 nucleotides.

In a specific embodiment, the term "standard hybridization conditions" refers to a Tm of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the Tm is 60°C; in a more preferred embodiment, the Tm is 65°C.

A DNA "coding sequence" or "encoding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to

include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

A coding sequence is "under the control"of or "operationally associated" with transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence. A "signal sequence" can be included before the coding sequence. This sequence encodes a "signal peptide", N-terminal to the polypeptide, that directs the host cell to transport the polypeptide to the cell surface or secrete the polypeptide into the media. The signal peptide is usually selectively degraded by the cell upon exportation. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear or circular DNA molecules, restriction fragments, viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

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Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

A coding sequence is "under the control"of or "operationally associated" with transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence. A "signal sequence" can be included before the coding sequence. This sequence encodes a "signal peptide", N-terminal to the polypeptide, that directs the host cell to transport the polypeptide to the cell surface or secrete the polypeptide into the media. The signal peptide is usually selectively degraded by the cell upon exportation. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

<u>Nucleic Acid Molecules Encoding Hybrid Proteins</u>

The invention relates to isolated nucleic acid molecules encoding recombinant allergen hybrid proteins. The invention further relates to a cell line stably containing a

recombinant nucleic acid molecule encoding a allergen hybrid protein, and capable of expressing such nucleic acid molecule to produce the hybrid protein. The nucleic acids can be generated from allergens, e.g., as listed in Table 8 and in certain patents and patent applications disclosed herein.

As a specific example, the present disclosure provides the complete nucleic acid sequence of a vespid venom protein. In particular, the present disclosure provides the nucleic acid sequence of a vespid Ag 5, in particular Ves v Ag 5 (SEQ ID NO: 14; see Fig. 1) and Pol a Ag 5 (SEQ ID NO:15; see Fig. 2). Also provided are the amino acid sequences of Ves v Ag 5 (SEQ ID NO: 16; see Fig. 1) and Pol a Ag5 (SEQ ID NO: 17; see Fig. 2).

In a specific embodiment, to obtain a nucleic acid molecule of the invention, DNA fragments are amplified by polymerase chain reaction (PCR) to amplify a fragment encoding a sequence comprising the allergen peptide epitope sequence or a scaffold protein. Oligonucleotide primers representing an allergen protein or scaffold protein of the invention can be used as primers in PCR. Generally, such primers are prepared synthetically. PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™).

Nucleic acids of the invention may also be obtained by cloning of restrictions fragments. Alternatively, nucleic acids of the invention may be obtained by recombination of nucleic acids in vivo or in vitro. In some instances recombination depends on sequence homology between the nucleic acids that participate in a recombination event, but in other instances the nucleic acids undergoing recombination need not contain significant homology, as is the case, for example, in "illegitimate" recombination events. One of ordinary will recognize recombination of nucleic acids may be an inter- or intramolecular event.

Alternatives to isolating the allergen proteins or scaffold DNA or cDNA include, but are not limited to, chemically synthesizing the gene sequence itself from the sequence provided herein.

The above methods are not meant to limit the methods by which DNA of the invention may be obtained.

The methods used to obtain a nucleic acid of the invention may lead to the insertion or deletion of nucleotides at junctions where nucleic acids are joined, by recombinant or other techniques. In one embodiment, nucleotides may be inserted or deleted

at the junction of a nucleic acid encoding an antigenic peptide and the nucleic acid encoding a scaffold protein. Such nucleic acids are fully within the scope of the invention. Accordingly, the invention encompasses hybrid proteins wherein amino acids have been inserted or deleted at the junction of a peptide epitope sequence and a scaffold protein sequence.

Nucleic acid sequence of the cloned hybrid protein, or starting materials thereof, can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. In the production of the nucleic acid encoding a hybrid protein, care should be taken to ensure that the modified nucleic acid remains within the same translational reading frame as the scaffold protein, uninterrupted by translational stop signals.

Additionally, the nucleic encoding an allergen peptide epitope sequence or scaffold protein can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, in vitro site-directed mutagenesis (Hutchinson *et al.*, 1978, J. Biol. Chem. 253:6551; Zoller and Smith, 1984, DNA 3:479-488; Oliphant *et al.*, 1986, Gene 44:177; Hutchinson *et al.*, 1986, Proc. Natl. Acad. Sci. U.S.A. 83:710), use of TAB® linkers (Pharmacia), etc. PCR techniques are preferred for site directed mutagenesis (see Higuchi, 1989, "Using PCR to Engineer DNA", in PCR Technology: Principles and Applications for DNA Amplification, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70).

A large number of vector-host systems known in the art may be used to express a DNA of the invention. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as various pBR322 derivatives, for example, pUC, CR, pGEX vectors, pmal-c, pFLAG, etc. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini.

In a preferred aspect of the invention, the PCR amplified nucleic acid molecules of the invention contain 3'-overhanging A-nucleotides, and can be used directly for cloning into a pCR vector with compatible T-nucleotide overhangs (Invitrogen Corp., San Diego, CA). However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and a DNA of the invention may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the DNA of the invention enables generation of multiple copies of the DNA. Thus, the DNA may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted sequences from the isolated recombinant DNA.

The nucleotide sequences encoding Ves v 5 polypeptide epitope sequences of SEQ ID NO: 1-13 and 93-95 are given respectively in SEQ ID NO: 18-30 and 96-98.

Expression of an Allergen Hybrid Protein

The nucleotide sequence coding for a hybrid protein or an immunomodulatory fragment, derivative or analog thereof, can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Such elements are termed herein a "promoter." Thus, the nucleic acid molecule encoding the hybrid protein is operationally associated with the promoter. An expression vector also preferably includes a replication origin. The necessary transcriptional and translational signals can also be supplied by the native gene encoding the allergen or scaffold protein and/or its flanking regions. Potential host-vector systems include but are not limited to mammalian cell systems, e.g., infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems, e.g., infected with virus (*e.g.*, baculovirus);

microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

In an alternative embodiment, a recombinant hybrid protein of the invention, or an immunomodulatory fragment, derivative or analog thereof, is expressed chromosomally, after integration of the hybrid protein coding sequence by recombination. In this regard, any of a number of amplification systems may be used to achieve high levels of stable gene expression (See Sambrook *et al.*, 1989, supra, at Section 16.28).

The cell into which the recombinant vector comprising the nucleic acid molecule encoding the hybrid protein is cultured in an appropriate cell culture medium under conditions that provide for expression of the hybrid protein by the cell. The expressed hybrid protein can then be recovered from the culture according to methods well known in the art. Such methods are described in detail, *infra*.

In a another embodiment, a hybrid protein can be expressed initially with amino acids that are subsequently cleaved from the hybrid protein. The sequences to be removed can be amino- or carboxyl-terminal to the hybrid protein sequences. The sequences may be removed either *in vivo* or *in vitro*. Preferably the sequences are removed by cleavage at a specific site by a protease, *e.g.*, signal peptidase, Factor Xa, Kex2 or a dipeptidyl amino peptidase. A recombinant DNA molecule encoding such a hybrid protein that includes a polypeptide to be cleaved by a protease comprises a sequence encoding the peptide to be cleaved from the hybrid protein joined in-frame to the coding sequence for a allergen hybrid.

In a specific embodiment, the hybrid proteins are expressed with an additional sequence comprising about six histidine residues, *e.g.*, using a pQE vector (QIAGEN, Chatsworth, CA). The presence of the histidine makes possible the selective isolation of recombinant proteins on a Ni-chelation column. Other such handles include, but are not limited to, FLAG, a myc tag, GST, etc.

In another embodiment, a periplasmic form of the hybrid protein (containing a signal sequence) can be produced for export of the protein to a yeast periplasm or into a culture medium. Export to the periplasm or into the medium can promote proper folding of the expressed protein.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinants (genetic recombination).

Expression of nucleic acid sequence encoding a hybrid protein, or an immunomodulatory fragment thereof, may be regulated by a second nucleic acid sequence so that the hybrid protein is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a hybrid protein may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control expression of the hybrid protein coding sequences include, but are not limited to, the CMV promoter, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff *et al.*, 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer *et al.*, 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.*, of a signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an nonglycosylated core protein

product. However, the enzyme protein expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in insect cells can be used to increase the likelihood of native glycosylation and folding of a heterologous allergen hybrid protein. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.

Vectors are introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell hybrid, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu *et al.*, 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut *et al.*, Canadian Patent Application No. 2,012,311, filed March 15, 1990).

Both cDNA and genomic sequences can be cloned and expressed.

It is further contemplated that the hybrid proteins of the present invention, or fragments, derivatives or analogs thereof, can be prepared synthetically, *e.g.*, by solid phase peptide synthesis.

Once the recombinant hybrid protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, size exclusion, and reverse phase chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

In a particular embodiment, a hybrid protein and fragments thereof can be engineered to include about six histidyl residues, which makes possible the selective isolation of the recombinant protein on a Ni-chelation column. In a preferred aspect, the proteins are further purified by reverse phase chromatography.

In another embodiment, the recombinant hybrid protein may include additional sequences that allow the hybrid protein to be targeted for affinity purification such as FLAG, MYC, or GST (glutathione-S-transferase). For example, antibody specific for the additional sequences of the hybrid protein can be immobilized on a solid support, *e.g.*, cyanogen bromide-activated Sepharose, and used to purify the hybrid protein. In another embodiment, a binding partner of the additional sequences, such as a receptor or ligand, can be immobilized and used to affinity purify the hybrid protein.

In one embodiment, the hybrid protein, preferably purified, is used without further modification, *i.e.*, without cleaving or otherwise removing any sequences that may be present in addition to the peptide epitope sequence and the scaffold protein. In a preferred embodiment, the hybrid protein can be used therapeutically, *e.g.*, to modulate an immune response.

In a further embodiment, the purified hybrid protein is treated to cleave and remove any sequences that may have been added to the scaffold protein. For example, where the hybrid protein has been prepared to include a protease sensitive cleavage site, the hybrid protein can be treated with the protease to cleave the protease specific site and release the hybrid protein. In a specific embodiment, the hybrid protein is cleaved by treatment with Factor Xa.

In particular embodiments, recombinant hybrid proteins of the present invention include but certainly are not limited to those comprising, as a vespid venom antigen, a Ves v 5 peptide of SEQ ID NO: 1-13 or 93-95.

In a particular embodiment, recombinant vespid venom hybrid proteins of the present invention include but certainly are not limited to those comprising, as a scaffold protein, Pol a 5 protein of SEQ ID NO: 17.

Hybrid proteins can contain altered epitope or scaffold, or both, sequences, in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a conservative amino acid substitution. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Manipulations of the recombinant hybrid protein may also be made at the protein level such as glycosylation, acetylation, phosphorylation, amidation, reduction and

carboxymethylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In a particular embodiment, the hybrid protein is expressed in an insect cell expression system, *e.g.*, using a baculovirus expression vector. In a preferred embodiment, the hybrid protein is expressed in yeast, *e.g.*, without limitation, Picchia pastoris, using appropriate expression systems. As pointed out above, these expression systems should yield "native" glycosylation and structure, particularly secondary and tertiary structure, of the expressed polypeptide.

Activity Assays With Hybrid Proteins of the Invention

Numerous assays are known in immunology for evaluating the immunomodulatory activity of an antigen. For example, the hybrid proteins can be tested for the ability to bind to antibodies specific for the allergen or the scaffold. Preferably, such antibodies that are detected in the diagnostic assay are of the IgG or IgE class. Hybrid proteins produced in eukaryotic expression systems, and particularly yeast cell expression systems, can have the correct structure for antibody binding. Hybrid proteins expressed in bacterial expression systems may not, and would thus require refolding prior to use in a diagnostic assay for antibody binding.

In another embodiment, the hybrid proteins of the invention can be tested in a proliferation assay for T cell responses. For such T cell response assays, the expression system used to produce the protein does not appear to affect the immunomodulatory activity of the protein. Generally, lymphocytes from a sensitized host are obtained. The host can be a mouse that has been immunized with an allergen, scaffold or hybrid protein, such as a vespid venom Ag 5 that has been produced recombinantly .

In a preferred embodiment, peripheral blood leukocytes are obtained from a human who is sensitive to the allergen. Using techniques that are well known in the art, T lymphocyte response to the protein can be measured in vitro. In a specific embodiment,

infra, T cell responses are detected by measuring incorporation of ^{3}H-thymidine, which increases with DNA synthesis associated with proliferation.

Cell proliferation can also be detected using an MTT assay (Mossman, 1983, J. Immunol. Methods 65:55; Niks and Otto, 1990, J. Immunol. Methods 130:140). Any method for detecting T cell proliferation known in the art can be used with the vespid protein produced according to the present invention.

Similarly, lymphokine production assays can be practiced according to the present invention. In one embodiment, lymphokine production can be assayed using immunological or co-stimulation assays (see, *e.g.*, Fehlner *et al.*, 1991, J. Immunol. 146:799) or using the ELISPOT technique (Czerkinsky *et al.*, 1988, J. Immunol. Methods 110:29). Alternatively, mRNA for lymphokines can be detected, *e.g.*, by amplification (see Brenner *et al.*, 1989, BioTechniques 7:1096) or *in situ* hybridization (see, *e.g.*, Kasaian and Biron, 1989, J. Immunol. 142:1287). Of particular interest are those individuals whose T cells produce lymphokines associated with IgE isotype switch events, *e.g.*, IL-4 and IL-5 (Purkeson and Isakson, 1992, J. Exp. Med. 175:973).

Thus, in a preferred aspect, the hybrid proteins produced according to the present invention can be used in *in vitro* assays with peripheral blood lymphocytes or, more preferably, cell lines derived from peripheral blood lymphocytes, obtained from allergen sensitive individuals to detect secretion of lymphokines ordinarily associated with allergic responses, *e.g.*, IL-4. Such assays may indicate which component or components of the hybrid protein are responsible for the allergic condition.

Therapeutic Uses of the Hybrid Protein and Nucleic Acid Vectors

The present invention provides a plentiful source of a hybrid protein, e.g., produced by recombinant techniques. Alternatively, a hybrid protein can be produced by peptide synthesis.

The invention contemplates use of hybrid proteins in therapeutic (pharmaceutical) compositions, for the use in the therapy of allergen-specific allergic conditions, treating allergen-specific allergic conditions, immune system related conditions, and modulating immune response in a mammal against an immunogen. In a specific embodiment, Ves v 5 and Pol a 5 hybrid proteins, or derivatives or analogs thereof, are

contemplated for use in diagnosis, therapy, treatment, and modulation of immune response according to the present invention.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to treat, and preferably increase by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, the ability of the immune system of a subject to combat effectively an immunogen. As further studies are conducted, information will emerge regarding appropriate dosage levels for modulation of immune system response towards an immunogen in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, will be able to ascertain proper dosing.

Therapeutic Methods

Therapeutic compositions of the invention (see, *infra*) can be used in immunotherapy, also referred to as hyposensitization therapy. Immunotherapy has proven effective in allergic diseases, particular insect allergy. Allergens are administered parenterally over a long period of time in gradually increasing doses. Such therapy may be particularly effective when the allergen or allergens to which the patient is sensitive have been specifically identified and the therapy is targeted to those allergen(s). However, this approach suffers the drawback of potentially precipitating an allergic reaction; especially anaphylaxis. Thus, the availability of hybrid proteins in large quantities is important for immunotherapy of allergy because they induce an effective IgG response against the allergen without an allergic reaction.

As discussed in the Background of the Invention, the presence of B cell epitopes on an allergen can cause an undesirable systemic reaction when the allergen is used for immunotherapy. Thus, a particular advantage of the invention is the capability to provide allergen polypeptides that do not cause undesirable systemic effects.

In one embodiment, one or more hybrid proteins can be injected subcutaneously to decrease the T cell response to the native molecule, *e.g.*, as described by Brine *et al.* (1993, Proc. Natl. Acad. Sci. U.S.A. 90:7608-12).

In another embodiment, one or more hybrid proteins can be administered intranasally to suppress allergen-specific responses in naive and sensitized subjects (see *e.g.*, Hoyne *et al.*, 1993, J. Exp. Med. 178:1783-88).

Administration of a hybrid protein of the invention is expected to induce a strong anti-allergen B cell (antibody), IgG response that will block IgE antibodies, and thus, have a therapeutic effect.

These results can also be achieved by administration of a vector that permits expression of the hybrid protein, *i.e.*, by gene therapy. Preferred vectors, particularly for cellular assays *in vitro* and *in vivo*, are viral vectors, such as lentiviruses, retroviruses, herpes viruses, adenoviruses, adeno-associated viruses, vaccinia virus, baculovirus, alphaviruses (especially Sindbis viruses and Semliki Forest viruses), and other recombinant viruses with desirable cellular tropism; and non-viral vectors. For gene therapy *in vivo* or *ex vivo*, a pharmaceutically acceptable vector is preferred, such as a replication incompetent viral vector. Pharmaceutically acceptable vectors containing the nucleic acids of this invention can be further modified for transient or stable expression. As used herein, the term "pharmaceutically acceptable vector" includes, but is not limited to, a vector or delivery vehicle having the ability to selectively target and introduce the nucleic acid into cells.

Thus, a gene encoding a functional or mutant protein or polypeptide domain fragment thereof can be introduced *in vivo*, *ex vivo*, or *in vitro* using a viral vector or through direct introduction of DNA. Expression in targeted tissues can be affected by targeting the transgenic vector to specific cells, such as with a viral vector or a receptor ligand, or by using a tissue-specific promoter, or both. Targeted gene delivery is described in PCT Publication No. WO 95/28494.

Viral vectors commonly used for *in vivo* or *ex vivo* targeting and therapy procedures are DNA-based vectors and retroviral vectors. Methods for constructing and using viral vectors are known in the art (*see, e.g.*, Miller and Rosman, BioTechniques 1992, 7:980-990). Preferably, the viral vectors are replication-defective, that is, they are unable to replicate autonomously in the target cell. Preferably, the replication defective virus is a minimal virus, *i.e.*, it retains only the sequences of its genome that are necessary for encapsidating the genome to produce viral particles.

DNA viral vectors include an attenuated or defective DNA virus, such as but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), alphavirus (especially Sindbis virus), and the like. Defective viruses that entirely or almost entirely lack viral genes are preferred.

Defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, a specific tissue can be specifically targeted. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt *et al.*, Molec. Cell. Neurosci. 1991, 2:320-330), defective herpes virus vector lacking a glyco-protein L gene, or other defective herpes virus vectors (PCT Publication Nos. WO 94/21807 and WO 92/05263); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet *et al.* (J. Clin. Invest. 1992, 90:626-630; *see also* La Salle *et al.*, Science 1993, 259:988-990); a defective adeno-associated virus vector (Samulski *et al.*, J. Virol., 1987, 61:3096-3101; Samulski *et al.*, J. Virol. 1989, 63:3822-3828; Lebkowski *et al.*, Mol. Cell. Biol. 1988, 8:3988-3996); and Alphavirus vectors, including Sindbis virus and Semliki Forest virus-based vectors (U.S. Patent No. 5,091,309; PCT Publication No. WO 98/44132; Schlesinger and Dubensky, Curr. Opin. Biotechnol. 1999, 5:434-9; Zaks et al., Nat. Med. 1999, 7:823-7).

Various companies produce viral vectors commercially, including, but not limited to, Avigen, Inc. (Alameda, CA; AAV vectors), Cell Genesys (Foster City, CA; retroviral, adenoviral, AAV, and lentiviral vectors), Clontech (retroviral and baculoviral vectors), Genovo, Inc. (Sharon Hill, PA; adenoviral and AAV vectors), Genvec (France; adenoviral vectors), IntroGene (Leiden, Netherlands; adenoviral vectors), Molecular Medicine (retroviral, adenoviral, AAV, and herpes viral vectors), Norgen (adenoviral vectors), Oxford BioMedica (Oxford, United Kingdom; lentiviral vectors), and Transgene (Strasbourg, France; adenoviral, vaccinia, retroviral, and lentiviral vectors).

In another embodiment, the vector can be introduced *in vivo* by lipofection, as naked DNA, or with other transfection facilitating agents (peptides, polymers, etc.). Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner, et. al., Proc. Natl. Acad. Sci. USA 1987, 84:7413-7417; Felgner and Ringold, Science 1989, 337:387-388; see Mackey, *et al.*, Proc. Natl. Acad. Sci. USA 1988, 85:8027-8031; Ulmer *et al.*, Science 1993, 259:1745-1748). Useful lipid compounds and compositions for transfer of nucleic acids are described in PCT Patent Publication Nos. WO 95/18863 and WO 96/17823, and in U.S. Patent No. 5,459,127. Lipids may be chemically coupled to other molecules for the purpose of targeting (see Mackey, *et. al.*,

supra). Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (*e.g.*, PCT Patent Publication No. WO 95/21931), peptides derived from DNA binding proteins (*e.g.*, PCT Patent Publication No. WO 96/25508), or a cationic polymer (*e.g.*, PCT Patent Publication No. WO 95/21931).

It is also possible to introduce the vector *in vivo* as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, *e.g.*, electroporation, microinjection, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (*see, e.g.*, Wu *et al.*, J. Biol. Chem. 1992, 267:963-967; Wu and Wu, J. Biol. Chem. 1988, 263:14621-14624; Canadian Patent Application No. 2,012,311; Williams *et al.*, Proc. Natl. Acad. Sci. USA 1991, 88:2726-2730). Receptor-mediated DNA delivery approaches can also be used (Curiel *et al.*, Hum. Gene Ther. 1992, 3:147-154; Wu and Wu, J. Biol. Chem. 1987, 262:4429-4432). U.S. Patent Nos. 5,580,859 and 5,589,466 disclose delivery of exogenous DNA sequences, free of transfection facilitating agents, in a mammal. Recently, a relatively low voltage, high efficiency *in vivo* DNA transfer technique, termed electrotransfer, has been described (Mir *et al.*, C.P. Acad. Sci. 1988, 321:893; PCT Publication Nos. WO 99/01157, WO 99/01158, and WO 99/01175).

Treatment of Immune System Related Diseases

As explained above, the present invention relates to hybrid proteins for treating immune system related diseases or disorders, or for modulating immune response in a mammal towards an immunogen. In particular, Applicant has discovered that the hybrid proteins of the invention have applications in modulating a subject's immune response to various immunogens, in a manner that elicits an immune response without eliciting an allergenic response. In a particular embodiment, hybrid proteins of the invention modulate a subject's immune system to have increased ability to combat pathogens and viruses including, but not limited to, HIV, Herpes Simplex virus, or papilloma virus. Such a method comprises administering to a subject a therapeutically effective amount of a pharmaceutical composition comprising a polypeptide encoded by an isolated nucleic acid molecule comprising a DNA molecule of the invention. Furthermore, it has been

discovered that the hybrid proteins, nucleic acids and vectors of the invention also have applications in treating an immune system related disease or disorder, or a symptom related thereto. As used herein, the phrase "immune system related disease or disorder" refers to a disease or disorder which evokes an immune response in a subject, or effects the ability of the immune system to respond to an immunogen. Examples of immune system related diseases or disorders which can be treated with agents and pharmaceutical compositions of the invention include, but are not limited to, a pathogenic disease or disorder; a viral disease or disorder, *e.g.* HIV, Herpes Simplex virus, or papilloma virus; or an autoimmune disease, *e.g.* arthritis or Lupus.

Moreover, the present invention extends to a method for treating an immune system related disease or disorder, or a symptom related thereto, comprising administering a therapeutically effective amount of a pharmaceutical composition for treating an immune system related disease or disorder to a subject. Hence, for example, should the immune system related disease or disorder involve HIV, a clinically significant change would, for example, involve an increase in white blood cell count in a subject to whom a pharmaceutical composition of the invention is administered relative to white blood cell count prior to administration. Other such examples of monitoring a clinically significant change in a subject will be readily apparent to one of ordinary skill in the art. Furthermore, as further studies are conducted, information will emerge regarding appropriate dosage levels for treating an immune system related disease or disorder, or a symptom related thereto in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, will be able to ascertain proper dosing. Examples of pharmaceutically acceptable compositions are described infra.

Pharmaceutically Acceptable Compositions

The *in vivo* therapeutic compositions of the invention may also contain appropriate pharmaceutically acceptable carriers, excipients, diluents and adjuvants. As used herein, the phrase "pharmaceutically acceptable" preferably means approved by a regulatory agency of a government, in particular the Federal government or a state government, or listed in the U.S. Pharmacopeia or another generally recognized pharmacopeia for use in animals, and more particularly in humans. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include mannitol, human serum albumin (HSA), starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium carbonate, magnesium stearate, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained-release formulations and the like.

Such compositions will contain an effective diagnostic or therapeutic amount of the active compound together with a suitable amount of carrier so as to provide the form for proper administration to the patient. While intravenous injection is a very effective form of administration, other modes can be employed, such as by injection, or by oral, nasal or parenteral administration.

The invention will be further clarified by the following examples, which are intended to be purely exemplary of the invention.

Example 1 Construction of Ag5 hybrid cDNAs

Primers 1-24 used in the Examples are listed in Table 1.

Ves v 5 EA and KR constructs were prepared by PCR amplification of Ves v 5 cDNA template (Lu *et al.*, 1993, J. Immunol. 150:2823) with the primers 1 (SEQ ID NO: 31) and 3 (SEQ ID NO: 33) or 2 (SEQ ID NO: 32) and 3 (SEQ ID NO: 33), respectively. Pol a 5 EA and KR constructs were prepared by PCR amplification of a Pol a cDNA template (Lu *et al.*, 1993, J. Immunol. 150:2823) with the primers 4 (SEQ ID NO: 34) and 6 (SEQ ID NO: 24) or 5 (SEQ ID NO: 35) and 6 (SEQ ID NO: 36), respectively. Each cDNA construct contained an EcoRI or XhoI site at the 5'terminus and an XbaI site at the 3'-terminus. cDNAs were cloned in the plasmid vector pPICZαA (Invitrogen Corp, San Diego, CA) as either EcoRI -XbaI or XhoI-XbaI fragments. Positive clones were identified by PCR. The sequences of recombinant Ag5 and hybrid cDNAs in pPICZαA were confirmed by DNA

sequencing of the inserts. Other constructs were prepared as described in King et al. (2001, J. Immunol. 166:6057-6065).

(i) PV1-46. The PV1-46 hybrid was constructed by joining amino-terminal sequences of Ves v 5 and carboxyl-terminal sequences of Pol a 5 at the peptide sequence EH, which is present at amino acids 47-48 and 49-50 of the respective proteins. The nucleotide sequence encoding the EH peptide in Ves v 5 is GAG CAC, which corresponds to a Bsi HKA I restriction enzyme cleavage site.

To facilitate construction of the PV1-46 hybrid, the natural DNA sequence (GAG CAT) encoding the Pol a 5 EH peptide at amino acids 49-50 was mutated to a Bsi HKA I site by a PCR overlap extension method (Ho *et al.*, 1989, Gene 77:51), as follows. A first step comprised two separate PCRs. In one PCR, primers 4 (SEQ ID NO: 34) and 8 (SEQ ID NO: 38) and were used to amplify DNA encoding residues 1-53 of Pol a 5 wherein the EH-encoding sequence was converted to a Bsi HKA I site. In a second PCR, primers 7 (SEQ ID NO: 37) and 6 (SEQ ID NO:36) were used to amplify DNA encoding residues 47-205 of Pol a 5 wherein the EH-encoding sequence was converted to a Bsi HKA I site. Both PCRs were performed with 1-40 ng Pol a cDNA as template and 50 pmole each of sense and anti-sense primers in 100 μl of PCR buffer containing 0.2 mM dNTPs and 5 units Taq polymerase. Cycling conditions were 0.5 min denaturation at 95°, 0.5 min annealing at 55° and 2 min extension at 72° for 35 cycles. The products of these two PCRs contained an overlap region. In the second step of the overlap extension procedure, the purified products of the first two reactions were mixed to served as the template for a third PCR with flanking primers 4 (SEQ ID NO: 34) and 6 (SEQ ID NO: 36), yielding a full length Pol a 5 with the EH-encoding sequence converted to a Bsi HKA I site.

Hybrid PV1-46 encoding cDNA was then prepared by ligation of the appropriate Bsi HKA I fragments from Ves v 5 and the modified Pol a 5 cDNAs into pPICZαA, as described above for Ag5 encoding cDNAs.

(ii) PV109-155. The PV109-155 hybrid was constructed by joining amino-terminal sequences of Ves v 5 and carboxyl-terminal sequences of Pol a 5 at the peptide sequence KY, which is present at amino acids 106-107 and 109-110 of the respective proteins. The KY peptides of both Ag 5s are encoded by the nucleotide sequence AAA TAT. To construct PV109-155, KY-encoding sequences of appropriate Ag5 or hybrid cDNAs

were mutated to an Apo I restriction enzyme cleavage site (AAA TTT) encoding a peptide sequence of KF. These single base mutations were made using the PCR overlap extension method (Ho et al., 1989, Gene 77:51) described in Example 1. In one set of reactions, the KY-encoding nucleotide sequence of PV1-155 cDNA was converted by performing the PCR overlap procedure with mutagenic primers 9 (SEQ ID NO: 39) and 10 (SEQ ID NO: 40). In a second set of reactions, the KY-encoding nucleotide sequence of Pol a 5 cDNA was converted by performing the PCR overlap procedure with mutagenic primers 11 (SEQ ID NO: 41) and 12 (SEQ ID NO: 42). Hybrid PV109-155 encoding cDNA was prepared by ligation of the appropriate fragments from Apo I digestions of converted Pol a 5 and converted PV1-155 encoding cDNAs into pPICZαA.

(iii) PV1-155 and PV156-204. Ves v 5 and Pol a 5 cDNAs have a common Eae I restriction site encoding amino acid residues 154-156. Hybrid PV156-204 and PV1-155 encoding cDNAs were prepared by ligation of the appropriate Eae I fragments of their parent cDNAs into pPICZαA.

(iv) PV1-8, PV1-18 and PV195-204. These hybrids were prepared by PCR with cDNA of Pol a 5 as the template. PV1-8 was prepared using primers 2 (SEQ ID NO: 32) and 6 (SEQ ID NO: 36). PV1-18 was prepared using primers 6 (SEQ ID NO: 36) and 13 (SEQ ID NO: 43). PV195-204 was prepared using primers 4 (SEQ ID NO: 34) and 14 (SEQ ID NO: 44). The hybrids were cloned into pPICZαA.

(v) PV1-24, PV1-32 , PV1-39, PV1-50, PV1-57 and PV1-70. These hybrids were constructed using the PCR overlap extension method given in Example 1 (Ho *et al.*, 1989, Gene 77:51). For PV1-24, first round PCRs were conducted using primers 1 (SEQ ID NO: 31) and 15 (SEQ ID NO: 45) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 16 (SEQ ID NO: 46) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 1 (SEQ ID NO: 31) and 6 (SEQ ID NO: 36) to yield PV1-24. For PV1-32, first round PCRs were conducted using primers 1 (SEQ ID NO: 31) and 18 (SEQ ID NO: 48) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 17 (SEQ ID NO: 47) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 1 (SEQ ID NO: 31) and 6 (SEQ ID NO: 36) to yield PV1-24. For PV1-39, first round PCRs were conducted using primers 2 (SEQ ID

NO: 32) and 19 (SEQ ID NO: 49) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 20 (SEQ ID NO: 50) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 2 (SEQ ID NO: 32) and 6 (SEQ ID NO: 36) to yield PV1-39. For PV1-50, first round PCRs were conducted using primers 2 (SEQ ID NO: 32) and 28 (SEQ ID NO: 58) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 27 (SEQ ID NO: 57) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 2 (SEQ ID NO: 32) and 6 (SEQ ID NO: 36) to yield PV1-50. For PV1-57, first round PCRs were conducted using primers 2 (SEQ ID NO: 32) and 30 (SEQ ID NO: 60) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 29 (SEQ ID NO: 59) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 2 (SEQ ID NO: 32) and 6 (SEQ ID NO: 36) to yield PV1-57. For PV1-76, first round PCRs were conducted using primers 2 (SEQ ID NO: 32) and 32 (SEQ ID NO: 62) with Ves v 5 cDNA as template and primers 6 (SEQ ID NO: 36) and 31 (SEQ ID NO: 61) with Pol a 5 cDNA as template. The two overlapping PCR products were then purified and used as template in a third PCR using flanking primers 2 (SEQ ID NO: 32) and 6 (SEQ ID NO: 36) to yield PV1-76. Hybrid cDNAs were cloned into pPICZαA.

(vi) PV22-32, PV115-125, PV142-150 and PV176-182. These constructs are hybrid Ag 5s wherein short Ves v 5 polypeptides replace homologous sequences in otherwise intact full length Pol a 5.

The Pol a 5 sequences were substituted with Ves v 5 sequences using the PCR overlap extension method given in Example 1 (Ho *et al.*, 1989, Gene 77:51). The template DNA used for the first set of two PCRs was the Pol a cDNA of Lu et al. (1993, J. Immunol. 150:2823). The upstream and downstream Pol a primers used in the PCR extension protocols were primers 4 (SEQ ID NO: 22) and 6 (SEQ ID NO: 24), respectively. Final products were cloned into pPICZαA.

The overlapping primer pairs encoding the inserted Ves v 5 sequences were as follows: *(a)* PV22-32– primers 17 (SEQ ID NO: 47) and 18 (SEQ ID NO: 48) *(b)* PV115-125– primers 21 (SEQ ID NO: 51) and 22 (SEQ ID NO: 52) *(c)* PV142-150– primers 23 (SEQ ID NO: 53) and 24 (SEQ ID NO: 54) and *(d)* PV176-182– primers 25 (SEQ ID NO:

55) and 26 (SEQ ID NO: 56). PCR reaction and cycling conditions were those described for PV1-46.

Table 1. Primers for preparation of Ves v and Pol a 5s and their hybrids.

Primer	Sequence (5' to 3')
1	CGTGAATTCAACAATTATTGTAAAATAAAA (SEQ ID NO: 31)
2	CGTCTCGAGAAAAGAAACAATTATTGTAAAATAAAA (SEQ ID NO: 32)
3	CGTTCTAGATTACTTTGTTTGATAAAGTTC (SEQ ID NO: 33)
4	CGTGAATTCGTTGATTATTGTAAAATAAAA (SEQ ID NO: 34)
5	CGTCTCGAGAAAAGAGTTGATTATTGTAAAATAAAA (SEQ ID NO: 35)
6	CGTTCTAGATTATTTTTTTGTATAAGGTAG (SEQ ID NO: 36)
7	GTAAGCGAGCACAATCGGTTT (SEQ ID NO: 37)
8	AAACCGATTGTGCTCGCTTAC (SEQ ID NO: 38)
9	GTAGCAAAATTTCAGGTTGGA (SEQ ID NO: 39)
10	TCCAACCTGAAATTTTGCTAC (SEQ ID NO: 40)
11	ACCGCAAAATTTCCAGTTGGA (SEQ ID NO: 41)
12	TCCAACTGGAAATTTTGCGGT (SEQ ID NO: 42)
13	CGTGAATTCAACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGAGAA (SEQ ID NO: 43)
14	CGTTCTAGATTACTTTGTTTGATAAAGTTCCTCATTCTTAAAATTTCCAGCTGG (SEQ ID NO: 44)
15	GGCACAATTCTTGCTCGGTTTAAGACTTCCATA (SEQ ID NO: 45)
16	TATGGAAGTCTTAAACCGAGCAAGAATTGTGCC (SEQ ID NO: 46)
17	CTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCGGTTGGTCCA (SEQ ID NO: 47)
18	TGGACCAACCGATACCACTACCTTATTACCGCAATTCGGTTTAAG (SEQ ID NO: 48)
19	TATGGTCTAACGAAACAAGAGAAAAAATTAATCGTA (SEC ID NO: 49)
20	TACGATTAATTTTTTCTCTTGTTTCGTTAGACCATA (SEC ID NO: 50)
21	TTAACAGGTAGCACGGCTGCTAAATACGATGATGTAGTCAGTCTA (SEQ ID NO: 51)
22	ATCATCGTATTTAGCAGCCGTGCTACCTGTTAACGCTATATTTG (SEQ ID NO: 52)

23 CCTAAGAAAAAGTTTTCGGGAAACGACTTTGCTAAAATTGGC (SEQ ID NO: 53)

24 GTCGTTTCCCGAAAACTTTTTCTTAGGATTAAAATCTTTCAC (SEQ ID NO: 54)

25 ATTCAAGAGAAATGGCACAAACATTACCTCATA (SEQ ID NO: 55)

26 TTTGTGCCATTTCTCTTGAATATATTTTAGAGA (SEQ ID NO: 56)

27 GAGCACAATGACTTTAGACAAAAA (SEQ ID NO: 57)

28 TTTTTGTCTAAAGTCATTGTGCTC (SEQ ID NO: 58)

29 AAAATTGCACGAGGGTTGGAAACA (SEQ ID NO: 59)

30 TGTTTCCAACCCTCGTGCAATTTT (SEQ ID NO: 60)

31 AATATGAAAAATTTGGTATGGAAC (SEQ ID NO: 61)

32 GTTCCATACCAAATTTTTCATATT (SEQ ID NO: 62)

Ag5- or hybrid-encoding cDNAs of the EA- or KR-series were digested, respectively, with restriction enzymes Eco RI or Xho I, and Xba I, then inserted into similarly cut pPICZα-A vector (Invitrogen, San Diego, CA). The recombinant plasmids were amplified in TOP10F' cells. The Ag 5-coding sequences of all recombinant plasmids were confirmed by DNA sequencing. The Ag 5 coding-sequences corresponded to the sequence data in Genbank (Accession number M98858 for Ves v Ag 5 and accession number M98857 for Pol a Ag 5), with the exceptions of two single-nucleotide differences observed for Ves v 5. These changes were at positions 579 and 587 and resulted, respectively, in a silent G to A mutation and a T to A substitution that resulted in a codon change of M to K at amino acid residue 196. The two nucleotide changes may represent insect polymorphism, rather than random mutations since the Ag 5 cDNAs used were prepared in the same manner as it was done previously (Lu *et al.*, 1993, J. Immunol. 150:2823).

Example 2 Expression and purification of Ag 5s and hybrids

Recombinant plasmids (1-2 μg) were linearized by cutting with the restriction enzyme Sac I then used to transform competent Pichia pastoris KM71 yeast cells (about 8 x 10^9 cells in 40 μl of 1M sorbitol) by electroporation. Transformed cells were diluted to 2 ml with 1M sorbitol and allowed to recover at 30° for 1 hr without shaking and for an additional hour with shaking at 200 rpm. Aliquots of 50 μl or 100 μl aliquots were then spread on 100 mm plates of YPDS medium containing 1.5 mg/ml Zeocin for selection of multi-copy

integrants (Invitrogen Manual). Selected clones were picked after 3-4 day incubation and screened by small scale expression to identify colonies producing hybrid protein. Small scale expression was carried out in 50 ml plastic tubes in the same manner as described below for large scale isolation but at 1/30 scale and the culture fluids were screened by SDS gel electrophoresis for secreted proteins.

Yeast cells from selected clones were grown in two 500 ml bottles, each containing 150 ml of pH 6.0 phosphate buffer containing yeast nitrogen base, biotin, glycerol and histidine at 30° with orbital shaking at 250 rpm to an A_{600nm} of 10-12. Cells were then collected by centrifugation and resuspended in 100 ml of similarly buffered medium containing methanol in place of glycerol. Incubation was continued at 30° with shaking at 250 rpm for 4-6 days with daily addition of 1 ml of 50% methanol.

Ag 5s or their hybrids were purified from the culture fluid concentrate by ion-exchange chromatography on SE-cellulose (Sigma) using a previously reported procedure (Monsalve *et al.*, 1999, Protein Expr. Purif. 16:410). About 70% of the main peak was pooled, desalted by reversed phase chromatography on C18 silica and lyophilized. Recombinant Ag 5s or hybrids were dissolved in 0.01 M ammonium acetate buffer (pH 4.6) and stored at 4°. Recombinant protein concentrations were determined from absorbance at 280 nm, using molar extinctions calculated from tyrosine and tryptophan contents. The yields of Ag 5s or hybrids typically ranged from 1 to 7 mg per 100 ml of 4-day cultures.

Recombinant Ag 5s or hybrids were characterized by SDS gel electrophoresis, N-terminal sequence analysis and MALDI mass spectrometry. CD spectra at 0.2 mg/ml of recombinant proteins in 0.01 M acetate buffer of pH 4.6 were taken in cells of 1mm path length in an AVIV 62DS spectrometer.

Example 3 Physico-chemical characterization of recombinant vespid Ag 5s and hybrids

The Ag5s and hybrid proteins expressed in yeast strain KM71contained a secretory signal peptide. The signal peptide was linked to the expressed protein via a peptide of KR or KREAEAEF sequence. These two types of proteins were designated as the KR- and EA-series, respectively. Upon secretion from the yeast cells, the signal peptide was

cleaved from the secreted protein at the KR sequence (Kex 2 protease site) or the two EA sequences (Ste 13 dipeptidyl amino peptidase sites) (Invitrogen Manual).

Recombinant proteins were isolated from culture fluid by ion exchange chromatography on SE-cellulose followed by reversed phase chromatography on C18-silica and characterized by SDS gel electrophoresis. (Fig. 6). Several hybrids showed a closely-spaced doublet with mobilities similar to that of natural Ves v 5. The doublets are consistent with the varying extents of processing at their N-terminal ends, as indicated by N-terminal sequencing of hybrids PV1-155 and PV156-204 and mass spectrometry data (Table 2).

Recombinant Ag 5s and hybrids showed nearly identical CD spectra as those of the natural Ag 5s (Fig. 7). The spectra of the natural Ves v 5 and the EA-Ves v 5, and those of EA-PV1-46, EA-PV1-155 and EA-PV156-204 showed the presence of minima at about 208 nm with a shoulder at 225 nm (Fig. 7). These features are indicative of an ordered feature (Yang *et al.*, 1986, Methods in Enzymology 130:208). Similar CD spectra were observed for the other hybrids listed in Table II (data are not shown). The CD spectrum of recombinant Ves v 5 from bacteria showed a minima at about 200 nm, which is indicative of a disordered structure (Monsalve *et al.*, 1999, Protein Expr. Purif. 16:410).

The recombinant Ag 5s and hybrids from yeast were freely soluble in acid or basic buffers, as were the natural Ag 5s. This is in contrast to recombinant vespid Ag 5s from bacteria, which were freely soluble only in acidic buffer.

Results of mass spectrometric analysis of Ag 5s and hybrids are given in Table 2. EA-series Ag 5s were cleaved efficiently at the Kex 2 site but showed variable cleavages at the two Ste 13 sites. Recombinant EA-series proteins, therefore, had amino-terminal sequences of EAEAEF and EAEF, where the EF sequence was encoded by the Eco R I site used to insert cDNA into the vector. These data were similar to results reported previously (Monsalve *et al.*, 1999, Protein Expr. Purif. 16:410).

The EAEAEF sequence of recombinant Ves v 5 is known to function as a strong hapten (Monsalve *et al.*, 1999, Protein Expr. Purif. 16:410). Therefore, Ag 5s were also expressed as KR-series hybrids. Cleavage of KR-series proteins at the Kex 2 site yielded recombinant proteins with the N-terminal sequence of the natural proteins. Mass spectrometry analysis of the KR-series proteins Ves v5, Pol a 5, and hybrids KR-PV1-24 and KR-PV1-46 showed that they were cleaved, with varied efficiencies, at the Kex2 site, and at

residues 2, 7, and 9 upstream of the Kex2 site. (Table 2.) The recombinant proteins of the KR-series were usually of slightly lower yields than those of the EA-series.

Table 2. Mass spectrometric data of recombinant vespid Ag 5s and hybrids.

Protein	Assumed sequence	Abundance[1]	Mass units	
			calc'd	found
EA-Ves v 5	EAEAEF-Vv	80%	23,954	23,947
	EAEF-Vv	20%	23,754	23,752
EA-Pol a 5	EAEAEF-Pa	100%	23,611	23,613
EA-PV1-18	EAEF-PV	43%	23,497	23,506
	EAEAEF-PV	36%	23,697	23,698
	REAEAEF-PV	21%	23,871	23,827
EA-PV1-18	EAEAEF-PV	100%	23,697	23,701
EA-PV1-32	EF-PV	60%	22,964	22,930
	EAEF-PV	40%	23,151	23,134
EA-PV1-46	EAEF-PV	53%	23,300	23,327
	EAEAEF-PV	47%	23,500	23,515
EA-PV1-46	EF-PV	10%	23,099	23,109
	EAEF-PV	50%	23,300	23,327
	EAEAEF-PV	40%	23,500	23,515
EA-PV1-155	EF-PV	53%	23,375	23,334
	EAEF-PV	47%	23,575	23,533
EA-PV22-32	EAEF-PV	55%	23,135	23,203
	EAEAEF-PV	45%	23,336	23,371
EA-PV115-125	EAEAEF-PV	100%	23,873	23,887
EA-PV142-150	EAEAEF-PV	100%	23,592	23,585
EA-PV156-204	EAEF-PV	59%	23,776	23,775
	EAEAEF-PV	41%	23,932	23,939
EA-PV195-204	EAEAEF-PV	70%	23,700	23,688
	REAEAEF-PV	30%	23,874	23,844

KR-Ves v 5	Vv5	90%	23,277	23,274
	EEGVSLEKR-Vv	10%	24,305	24,298
KR-Ves v 5	Vv	95%	23,277	23,284
	EEGVSLEKR-Vv	5%	24,305	24,300
KR-Pol a 5	Pa	20%	22,934	22,951
	EEGVSLEKR-Pa	80%	23,962	23,992
KR-Pol a 5	Pa	10%	22,934	22,935
	EEGVSLEKR-Pa	90%	23,962	23,962
KR-PV1-24	PV	85%	22,903	22,897
	EEGVSLEKR-PV	15%	23,931	23,933
KR-PV1-46	PV	70%	22,823	22,834
	KR-PV	30%	23,107	23,157
KR-PV1-46	PV	60%	22,823	22,834
	KR-PV	40%	23,107	23,157

[1]Protein abundance was estimated from peak heights of samples in mass spectra. N-terminal sequences For of EA-Ves v 5, EA-Pol a 5, EA-PV3PV156-204 and EA- VP3PV1-155 samples of EA series, their assumed sequences were confirmed by Edman degradation.

Results of two preparations are shown for each of EA-PV1-18, EA-PV1-46, KR-Ves v 5, KR-Pol a and KR-PV1-46.

Amino terminal peptides have been assigned SEQ ID NO: as follows; EAEAEF [SEQ ID NO: 89]; EAEF [SEQ ID NO: 90]; REAEAEF [SEQ ID NO: 91] and EEGVSLEKR [SEQ ID NO: 92].

Example 4 ELISA studies

ELISA was performed in 96-well plates in the wells coated with 4 μg/ml Ag 5 in 0.05 M Tris-HCI buffer of pH 8. Bound IgG_1 was detected with 2 μg/ml biotinylated goat anti-mouse IgG (γ1 specific) followed with 2 μg/ml avidin-peroxidase conjugate (King *et al.*, 1995, J. Immunol 154:577). Antibody concentrations of sera samples were determined by comparison of their ELISA data with that of an immuno-affinity purified sample of Ves v 5-specific antibody.

Example 5 Ves v 5-specific B cell epitopes of hybrids

Murine polyclonal antibodies specific for natural Ves v 5 were isolated from BALB/c sera by affinity chromatography on Ves v 5-specific immunosorbent and were

depleted of Pol a 5-cross-reacting antibodies by passage through Pol a 5-specific immunosorbent. The immunosorbents were prepared with CNBr activated Sepharose 2B (Pharmacia). Murine monoclonal antibodies specific for Ves v 5 were obtained as described (King *et al.*, 1987, Mol. Immunol 24:857).

Ves v 5-specific B cell epitopes were detected by hybrid-inhibition of binding of mouse Ves v 5-specific antibodies to solid-phase Ves v 5. Both EA- and KR-Ves v 5 were tested as solid phase antigen with similar results. Five samples of mouse antisera were tested; three were from BALB/c strains and one each from ASW/sn and P/J strains. Results using one BALB/c serum sample are shown in Fig. 8A. At the highest concentration of 50 or 500μg/ml inhibitor tested, the two N-terminal hybrids EA-PV1-46 and EA-1-155 showed maximal inhibition approaching 100%, as did EA- or KR-Ves v 5. Two other N-terminal hybrids KR-PV1-24 and EA-PV1-32 had maximal inhibition of about 60% and the shortest N-terminal hybrid, EA-PV1-18, had maximal inhibition of about 20%. The C-terminal hybrid EA-PV156-204 had maximal inhibition of about 15%. Similar results were obtained for results of inhibition ELISA using antisera from ASW/sn (Fig. 8B) and P/J (Fig. 8C) mice.

Ves v 5-specific B cell epitopes were also detected by inhibition analyses with sera from six yellow jacket sensitive patients. The data from three patients are shown in Fig. 9A-C. The results were similar to those obtained with mouse IgGs.

The results of the ELISA inhibition studies using both mouse and human antisera indicated the immunodominance of the N-terminal region of Ves v 5.

The observed inhibition by the hybrids was not due to cross-reacting epitopes of the Pol a 5 portion of the molecule as the sample of Ves v 5-specific antibodies used for inhibition studies in BALB/c mice was depleted of Pol a 5-cross-reactive antibodies and no inhibition by Pol a 5 was detected (Fig. 8A). The high concentrations of hybrids required for half maximal inhibition relative to that of Ves v 5 did not reflect that the epitopes of the hybrids lacked the native structure of Ves v 5 as the recombinant Ves v 5 from bacteria that lacked the native structure did not show any inhibition (data not shown).

The difference in the inhibitory activities of Ves v 5 and hybrids was probably related to their epitope densities. Epitope density is known to influence strongly the affinity constant of a multivalent antigen and a bivalent antibody (Hornick and Karush, 1972, Immunochemistry 9:325; Crothers and Metzger, 1972, Immunochemistry 9:341).

The data in Figs. 8 and 9 suggested that the amino terminal portion of Ves v 5 includes the immunodominant B cell epitopes of Ves v 5. This finding was confirmed by tests with a panel of 17 monoclonal antibodies specific for Ves v 5 (King *et al.*, 1987, Mol. Immunol 24:857). These monoclonal antibodies were specific for the natural Ves v 5 and recombinant proteins from yeast, but they did not bind the denatured form of recombinant Ves v 5 from bacteria (data not shown). ELISA results showed that one monoclonal antibody bound EA-Ves v 5 and EA-PV1-46 with similar affinity and maximal binding and it did not bind any of the other N- or C-terminal hybrids (Fig. 10A). Four other monoclonal antibodies showed greatly reduced maximal binding to EA-PV1-46 but no binding to any of the shorter N-terminal hybrids; the data for one such antibody are given in Fig. 10B. Lastly, one monoclonal antibody showed greatly reduced binding to EA-PV1-32 and EA-PV 1-46 and moderate binding to EA-PV1-18 and EA-PV 1-24 (Fig. 10C). These data show that six of the 17 monoclonal antibodies tested were specific for the N-terminal region of Ves v 5.

Example 6 Immune responses to hybrids

Groups of 3 or 4 female BALB/c mice were given biweekly intraperitoneal injections of 2 µg immunogen and 1 µg alum in 0.2 ml of phosphate buffered saline. Ag 5 or hybrid specific sera were collected at week 5 or later. Similar antibody levels were observed for sera collected at weeks 5, 7, and 9.

Mice immunized with hybrids produced antibodies specific for the hybrid, Pol a 5 and Ves v 5. The antibody levels of sera samples were measured before and after absorption with Pol a 5 to determine their specificity for Ves v 5. These data are summarized in Table 3A. Mice immunized with natural, EA- or KR-Ves v 5 gave nearly the same antibody responses, and only those of the KR-Ves v 5 are given Table 3A. EA-PV1-46 gave a higher antibody response in set A mice than KR-PV1-46 did in set B mice. This difference may be due to the different sets of mice used. EA-PV1-18 was used in both sets of experiments, and it gave higher antibody response in set A mice than that in set B mice.

Comparison of antibody levels in the N-terminal hybrid-specific sera samples in Table 3, before and after Pol a 5 absorption, indicated that 30-80% of the antibodies were specific for Ves v 5 when tested on solid-phase Ves v 5, and these values were less when tested on solid-phase hybrid. The higher contents of Ves v 5-specific antibodies detected on

solid-phase Ves v 5 than those on solid-phase hybrid suggest that the majority of hybrid-specific antibodies recognize overlapping regions of Ves v 5 and Pol a 5 in the hybrid. The data in set A of Table 3A indicated that of the three N-terminal hybrids, PV1-155 was as immunogenic as Ves v 5 was, PV1-46 was half as imunogenic as Ves v 5 and PV1-18 was about 1/9th as immunogenic as Ves v 5. The data in set B indicate that PV1-46 and 1-32 were more immunogenic than PV1-24 and 1-18. The data from both sets suggest that the longer N-terminal hybrids PV1-46 and 1-32 stimulate higher contents of Ves v 5-specific antibodies and lower contents of Pol a 5- specific antibodies than the two shorter hybrids PV1-24 and 1-18 did.

Table 3A

Murine antibody responses to vespid antigen 5s and hybrids

SET	Immunogen[1]	mg/ml specific IgG in sera by ELISA on solid-phase[2,3]		
		EA-Ves v 5	EA-Pol a 5	Hybrid
A	KR-Ves v 5	8.9 (8.5)	0.6	-
	KR-Pol a 5	2.8 (1.0)	7.0	-
	EA-PV1-155	12.0	0.7	-
	EA-PV1-46	4.2 (3.5)	1.9	7.6 (5.6)
	EA-PV1-18	1.0 (0.8)	6.9	6.9 (0.7)
	EA-PV156-204	1.6 (0.6)	10.0	2.6 (0.3)
	EA-PV195-204	1.3 (0.4)	14.0	10.0 (0.3)
B	KR-Ves v 5	15.0 (14.0)	0.2	-
	KR-PV1-46	0.6 (0.5)	1.0	2.7 (3.0)
	EA-PV1-32	0.9 (0.7)	4.3	8.0 (3.2)
	KR-PV1-24	0.4 (0.3)	4.2	6.5 (0.9)
	EA-PV1-18	0.4 (0.3)	4.5	5.3 (0.7)

1. Sera were collected on week 7, after 3 biweekly ip injections of immunogen. Sets A and B studies were made at separate occasions.
2. Antibody concentration was estimated from reciprocal sera concentration required to give an absorbance change of 1.0 in 30 minutes. Under the conditions used, this change corresponded to a 0.1µg/ml solution of purified Ves v 5 - specific antibody. The estimated antibody concentrations varied by about 40 % on repeat measurements.
3. Values in parenthesis were obtained after absorption of $^1/_{500}$ diluted sera with 0.2 mg/ml EA-Pol a 5.

The results shown in Table 3A indicate the B cell epitope of Ves v 5 is in its N-terminal region. Additional hybrids of Ves v 5 and Pol a 5 were prepared and tested for immunogenicity in mice as described above, to delineate the N and the C-terminal limits of the dominant B cell epitope region. Results are given in Table 3B, which lists the IgG1 content specific for Ves v, Pol a or hybrid, and percent of specific IgG1 remaining after absorption with Pa.

Hybrid PV1-8 with the lowest Ves v content did not induce Ves v-specific antibody response. All other hybrids induced 0.4-4.5 mg/ml of Ves v-specific Ab with the exception of PV22-32. Hybrids with Ves v contents <PV1-32 are moderately specific for Ves v response, as 34-81% of their Ves v-specific antbody and 15-27% of their hybrid-specific antibodies were not absorbed by Pol a 5. Hybrids with Ves v contents >PV1-39 are more specific, as 66-96% of their Ves v 5-specific antibody and 91-100% of their hybrid-specific antibody were not absorbed by Pol a 5. These results together suggest the C-terminal limit of the dominant epitope region is between residues 32-39.

Hybrids with Ves v contents of <PV1-32 show 2-4 mg/ml of Pol a-specific antibody, and hybrids with Ves v contents of >PV1-39 showed 0.04-1.34 mg/ml of Pol a-specific antibody. As the Ves v content of hybrids was increased from PV1-32 to 1-76, there was a progressive decrease of Pol a-specific response. These results together suggest the C-terminal limit of the dominant epitope extends beyond residues 39, as suggested by considerations of the Ves v-specific response to hybrids.

The lack of Ves v-specific antibody response of PV1-8 and 22-32 as compared to the response of PV1-32 suggests the N-terminal limit of the dominant epitope region to be within residues 9-21.

Table 3B. Murine antibody responses to vespid antigen 5s and hybrids

Construct	Groups of mice	Ves v 5 specific IgG1; % Ves v	Pol a 5 specific IgG1	Hybrid specific IgG1; % Ves v
Pol a 5	1	1.80 mg/ml; 64%	4.50 mg/ml	
Ves v 5	4	10.7 ± 3.2 mg/ml 104 ±15%	0.2 ±0.1 mg/ml	
PV1-8	1	0	8.2 mg/ml	
PV1-18	4	0.6 ±0.44 mg/ml; 68 ±14%	4.1 ±2.0 mg/ml	7.5 ±4.5 mg/ml; 27 ±26%

PV1-24	2	0.35 ±0.06 mg/ml; 81 ±17%	2.26 ±0.50 mg/ml	5.86 ±1.30 mg/ml; 20 ±12%
PV1-32	3	0.52 ±0.39 mg/ml; 34 ±25%	3.77 ±1.89 mg/ml	6.82 ±3.46 mg/ml; 15 ±6%
PV1-39	2	4.45 ±0.70 mg/ml; 89 ±27%	1.72 ±0.06 mg/ml	8.25 ±1/87 mg/ml; 76 ±23%
PV1-46	3	2.29 ±3.41 mg/ml; 86 ±14%	1.18 ±0.96 mg/ml	7.57 ±7.33 mg/ml; 91 ±9%
PV1-50	1	1.01 mg/ml; 94%	0.44 mg/ml	11.22 mg/ml; 90%
PV1-57	1	0.67 mg/ml; 96%	0.22 mg/ml	11.88 mg/ml; 85%
PV1-76	1	1.32 mg/ml; 92%	0.04 mg/ml	11.88 mg/ml; 92%
PV22-32	1	0.04 mg/ml; 0%	4.88 mg/ml	6.31 mg/ml; 6%

Data are from averages of week 7 bleedings from 1-4 groups of 4 mice.
% Ves v refers to antibody content after absorption with Pol a 5

Example 7 T cell response

Proliferation assays were performed with spleen cells from mice immunized with vespid antigen 5 or hybrid to study the specificity of T cell responses. Assays were performed in triplicate with spleen cells pooled from 2 to 3 mice, 10 days after 5 biweekly immunizations. Spleen cells (4×10^5) were cultured with test antigen in 0.2 ml of culture medium at 37° and 5% CO_2. Tritiated thymidine (1 μCi) was added on day 3, and the thymidine uptake was determined on day 4. The results were expressed as stimulation index values.

Results showed that the hybrids EA-PV1-46, EA-PV1-155 and EA-PV156-204 induced hybrid-specific as well as vespid antigen 5-specific T cell responses (Table 4). The data indicated that the best proliferative responses were obtained when the stimulating antigen was the immunogen. This is apparent from comparing the maximal stimulation index values at the highest antigen concentration of 100 μg/ml tested, and from comparing the lowest antigen concentration required for a stimulation index value of 4.

Table 4

Vespid antigen 5 or hybrid stimulated proliferation of murine spleen cells

Spleen cells specific for	EA-Ves v5	Stimulating Ag EA-Pol a 5	EA-hybrid
	Stimulation Index at 100 μg/ml Ag		
KR-Ves v 5	8.2	1.5	-
KR-Pol a 5	2.2	6.3	-
EA-PV1-155	6.1	2.2	5.0
EA-PV1-46	6.0	8.0	13.5
EA-PV1-18	2.3	5.0	6.1
EA-PV156-204	4.1	4.2	6.8
EA-PV195-204	1.7	8.6	4.1
	μg/ml Ag for stimulation index of 4		
KR-Ves v 5	2.6	>100	-
KR-Pol a 5	>100	16	-
EA-PV1-155	11	>100	0.54
EA-PV1-46	20	2.2	0.26
EA-PV1-18	>100	47	19
EA-PV156-204	60	70	2.3
EA-PV195-204	>100	8	82

Background proliferation of spleen cells showed ^{3}H-thymidine uptake of 400-900 cpm.

Example 8 Allergenicity of recombinant vespid Ag 5s and hybrids in patients

Allergenicity was determined by histamine release assay from basophils of 10 yellow jacket sensitive patients, following challenge with Ag 5 or hybrids (Colombo *et al.*, 1995, J Allergy Clin. Imm. 95:565). The patients/results shown in Table 5 are divided into two groups. Group A patients (n=7) were about 1000 times more sensitive to Ves v 5 than to Pol a 5; Group B patients (n=3) were about equally sensitive to both antigen 5s.

Table 5.

Summary of histamine release data of hybrids

	Reciprocal Activity Relative to Ves v 5					
	Group A			Group B		
Allergen	No. of patients	Mean	Range	No. of patients	Mean	Range
Ves v 5	7	1	1	3	1	1
Pol a 5	7	1154	330-5500	3	0.7	0.2 - 2
PV1-155	3	1	1-2	2	1	1
PV1- 46	5	126	13-3300	2	0.7	0.1 - 5
PV1- 18	3	583	12-5000	2	24	3.0 - 200

PV22-32	3	3207	2000-5000	2	6	6-20
PV115-125	3	3207	2000-5000	2	5	2-15
PV142-150	3	3000	2700-5000	2	5	2-15
PV156-204	6	1139	1000-3000	3	3	0.4 - 70
PV195-204	3	3207	50-5000	2	32	20.0 - 50

The complete data from one patient of each group are given in Figure 11.

Of the three N-terminal hybrids tested, EA-PV1-155 showed no decrease in allergnenicity. EA-PV1-46 and 1-18 showed geometric mean reductions of 126- and 583-fold respectively in group A patients, and 0.7- and 24-fold decreases respectively in group B patients. The two C-terminal hybrids EA-PV156-204 and 195-204 had reductions of 1139- and 3207-fold in group A patients respectively and 3- and 32-fold in group B patients respectively.

The different extents of reduction in allergenicity of the N- and C-terminal hybrids reflect both their IgE antibody concentration and their epitope density. The inhibition ELISA data in Figure 6 suggest a higher concentration of human IgG antibodies for the N-terminal region of Ves v 5 than those for the C-terminal region and this is likely also the case for IgE antibodies. Another contributing factor to the greater reduction in allergenicity of the C-terminal hybrid EA-PV156-204 as compared to the N-terminal hybrid EA-PV1-46 is probably due to its decreased epitope density as the C-terminal hybrid has fewer surface accessible residues of Ves v 5 than the N-terminal hybrid does. Similarly, the greater reduction in allergenicity of the shorter N- or C-terminal hybrids, PV1-18 or PV195-204, as compared to their respective longer ones also reflects the influence of epitope density.

The allergenicity of recombinant Ves v 5 from bacteria was compared with those of the natural Ves v and the recombinant Ves v 5 from yeast. In three patients tested, the recombinant protein from bacteria was about 103 times less potent than the natural protein or the recombinant protein from yeast (data not shown). These data confirm previous observations that the majority of B cell epitopes for allergens are dependent on the conformation of the native allergen (King *et al.*, 2000, Int Arch Allergy 123:99).

The decrease in allergenicity of the recombinant Ves v 5 from bacteria, was due to loss of the conformation dependent B cell epitopes as the CD spectrum of the recombinant protein from bacteria showed it to have a disordered structure. However, the

decrease in allergenicity of the hybrid protein PV1-46 or PV156-204 was due to reduction of the number and density of Ves v 5-specific epitopes, as its CD spectrum indicated it had an ordered structure similar to that of Ves v 5. The reduction of the number and density epitopes of the hybrid PV1-46 and PV156-204 is in agreement with the B cell epitope and immunogenicity data given in Examples 5-7.

Example 9 Crystallization of recombinant Ves v 5

Crystals of Ves v 5 was grown by the vapor diffusion technique at 25°C. For crystallization, 5 μl of 5 mg/ml Ves v 5 was mixed with 5 μl of 18% PEG 6000, 0.1 M sodium citrate, pH 6.0 and equilibrated against 1 ml of 18% PEG 6000, 0.1 M sodium citrate, pH 6.0. X-ray diffraction data was collected at 100K from native Ves v 5 crystals and after incorporation of heavy-atom derivatives and used to solve the three-dimensional structure of Ves v 5. The atomic coordinates and structure factors of Ves v 5 have been deposited in the Protein Data Bank (PDB) with the accession number Q05110. The atomic coordinates of Ves v 5 are given in Table 6.

Table 6. Ves v 5 crystal coordinates

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ATOM     21  OE2 GLU     3      23.681  46.586  20.793  1.00 37.45   APEP
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ATOM	139	O	GLY	18	30.510	33.622	35.195	1.00	21.83	APEP
ATOM	140	N	VAL	19	31.974	32.735	33.743	1.00	16.65	APEP
ATOM	141	CA	VAL	19	32.212	33.989	33.040	1.00	15.58	APEP
ATOM	142	CB	VAL	19	33.516	33.896	32.222	1.00	15.68	APEP
ATOM	143	CG1	VAL	19	33.884	35.254	31.649	1.00	13.84	APEP
ATOM	144	CG2	VAL	19	34.633	33.364	33.108	1.00	15.09	APEP
ATOM	145	C	VAL	19	31.045	34.361	32.115	1.00	14.11	APEP
ATOM	146	O	VAL	19	30.622	33.562	31.278	1.00	14.03	APEP
ATOM	147	N	HIS	20	30.528	35.577	32.265	1.00	11.37	APEP
ATOM	148	CA	HIS	20	29.410	36.020	31.444	1.00	11.65	APEP
ATOM	149	CB	HIS	20	29.094	37.493	31.704	1.00	12.93	APEP
ATOM	150	CG	HIS	20	27.721	37.900	31.264	1.00	13.85	APEP
ATOM	151	CD2	HIS	20	26.597	38.156	31.974	1.00	15.96	APEP
ATOM	152	ND1	HIS	20	27.392	38.102	29.941	1.00	15.59	APEP
ATOM	153	CE1	HIS	20	26.126	38.466	29.853	1.00	15.25	APEP
ATOM	154	NE2	HIS	20	25.620	38.506	31.072	1.00	17.34	APEP
ATOM	155	C	HIS	20	29.679	35.811	29.961	1.00	11.56	APEP
ATOM	156	O	HIS	20	30.783	36.054	29.467	1.00	9.12	APEP
ATOM	157	N	THR	21	28.650	35.355	29.260	1.00	12.15	APEP
ATOM	158	CA	THR	21	28.739	35.090	27.828	1.00	12.76	APEP
ATOM	159	CB	THR	21	27.349	34.686	27.287	1.00	13.90	APEP
ATOM	160	OG1	THR	21	27.016	33.387	27.792	1.00	14.96	APEP
ATOM	161	CG2	THR	21	27.336	34.658	25.756	1.00	13.84	APEP
ATOM	162	C	THR	21	29.294	36.278	27.025	1.00	12.07	APEP
ATOM	163	O	THR	21	30.102	36.090	26.111	1.00	8.89	APEP
ATOM	164	N	ALA	22	28.873	37.490	27.380	1.00	10.72	APEP
ATOM	165	CA	ALA	22	29.312	38.698	26.693	1.00	11.63	APEP
ATOM	166	CB	ALA	22	28.311	39.816	26.925	1.00	12.20	APEP
ATOM	167	C	ALA	22	30.706	39.156	27.102	1.00	13.47	APEP
ATOM	168	O	ALA	22	31.200	40.178	26.621	1.00	13.74	APEP
ATOM	169	N	CYS	23	31.332	38.410	28.006	1.00	14.12	APEP
ATOM	170	CA	CYS	23	32.683	38.715	28.460	1.00	14.19	APEP
ATOM	171	C	CYS	23	33.564	37.670	27.793	1.00	12.16	APEP
ATOM	172	O	CYS	23	34.725	37.909	27.497	1.00	13.84	APEP
ATOM	173	CB	CYS	23	32.782	38.599	29.995	1.00	12.96	APEP
ATOM	174	SG	CYS	23	34.454	38.855	30.695	1.00	14.19	APEP
ATOM	175	N	LYS	24	32.987	36.501	27.561	1.00	13.18	APEP
ATOM	176	CA	LYS	24	33.697	35.405	26.917	1.00	14.00	APEP
ATOM	177	CB	LYS	24	32.894	34.109	27.048	1.00	13.62	APEP
ATOM	178	CG	LYS	24	33.111	33.347	28.334	1.00	13.30	APEP
ATOM	179	CD	LYS	24	32.593	31.929	28.193	1.00	14.90	APEP
ATOM	180	CE	LYS	24	31.656	31.540	29.311	1.00	15.48	APEP
ATOM	181	NZ	LYS	24	32.009	30.188	29.830	1.00	21.39	APEP
ATOM	182	C	LYS	24	33.853	35.742	25.446	1.00	13.93	APEP
ATOM	183	O	LYS	24	34.917	35.578	24.861	1.00	14.28	APEP
ATOM	184	N	TYR	25	32.767	36.219	24.857	1.00	16.64	APEP
ATOM	185	CA	TYR	25	32.737	36.585	23.448	1.00	17.22	APEP
ATOM	186	CB	TYR	25	31.736	35.684	22.719	1.00	18.12	APEP
ATOM	187	CG	TYR	25	31.716	34.245	23.217	1.00	16.13	APEP
ATOM	188	CD1	TYR	25	30.600	33.727	23.879	1.00	18.60	APEP
ATOM	189	CE1	TYR	25	30.574	32.404	24.332	1.00	15.87	APEP
ATOM	190	CD2	TYR	25	32.810	33.403	23.021	1.00	16.98	APEP
ATOM	191	CE2	TYR	25	32.794	32.081	23.469	1.00	14.73	APEP
ATOM	192	CZ	TYR	25	31.677	31.590	24.120	1.00	16.64	APEP

ATOM	193	OH	TYR	25	31.661	30.283	24.566	1.00	19.74	APEP
ATOM	194	C	TYR	25	32.339	38.060	23.336	1.00	18.24	APEP
ATOM	195	O	TYR	25	31.155	38.404	23.332	1.00	17.58	APEP
ATOM	196	N	GLY	26	33.340	38.929	23.250	1.00	19.90	APEP
ATOM	197	CA	GLY	26	33.086	40.358	23.182	1.00	22.78	APEP
ATOM	198	C	GLY	26	32.536	40.927	21.886	1.00	25.12	APEP
ATOM	199	O	GLY	26	32.260	42.125	21.815	1.00	26.30	APEP
ATOM	200	N	SER	27	32.362	40.092	20.867	1.00	26.19	APEP
ATOM	201	CA	SER	27	31.855	40.570	19.583	1.00	26.72	APEP
ATOM	202	CB	SER	27	32.960	40.435	18.522	1.00	25.95	APEP
ATOM	203	OG	SER	27	32.457	40.041	17.259	1.00	24.78	APEP
ATOM	204	C	SER	27	30.586	39.839	19.139	1.00	26.86	APEP
ATOM	205	O	SER	27	30.159	38.878	19.774	1.00	25.87	APEP
ATOM	206	N	LEU	28	29.979	40.312	18.053	1.00	29.54	APEP
ATOM	207	CA	LEU	28	28.766	39.695	17.518	1.00	30.96	APEP
ATOM	208	CB	LEU	28	27.793	40.769	17.021	1.00	33.13	APEP
ATOM	209	CG	LEU	28	28.127	42.217	17.391	1.00	34.56	APEP
ATOM	210	CD1	LEU	28	29.022	42.812	16.319	1.00	34.22	APEP
ATOM	211	CD2	LEU	28	26.843	43.030	17.551	1.00	34.12	APEP
ATOM	212	C	LEU	28	29.142	38.769	16.365	1.00	30.72	APEP
ATOM	213	O	LEU	28	28.277	38.224	15.673	1.00	31.18	APEP
ATOM	214	N	LYS	29	30.448	38.602	16.176	1.00	30.29	APEP
ATOM	215	CA	LYS	29	31.008	37.759	15.124	1.00	29.17	APEP
ATOM	216	CB	LYS	29	32.490	38.102	14.937	1.00	31.20	APEP
ATOM	217	CG	LYS	29	33.016	37.866	13.534	1.00	32.99	APEP
ATOM	218	CD	LYS	29	34.528	37.785	13.521	1.00	34.25	APEP
ATOM	219	CE	LYS	29	35.150	39.121	13.885	1.00	35.23	APEP
ATOM	220	NZ	LYS	29	35.686	39.098	15.273	1.00	37.84	APEP
ATOM	221	C	LYS	29	30.867	36.269	15.444	1.00	27.53	APEP
ATOM	222	O	LYS	29	31.446	35.772	16.413	1.00	25.87	APEP
ATOM	223	N	PRO	30	30.104	35.530	14.621	1.00	27.59	APEP
ATOM	224	CD	PRO	30	29.362	36.011	13.442	1.00	26.03	APEP
ATOM	225	CA	PRO	30	29.905	34.091	14.840	1.00	25.87	APEP
ATOM	226	CB	PRO	30	28.982	33.675	13.694	1.00	25.48	APEP
ATOM	227	CG	PRO	30	28.330	34.949	13.245	1.00	24.87	APEP
ATOM	228	C	PRO	30	31.182	33.253	14.871	1.00	25.41	APEP
ATOM	229	O	PRO	30	32.061	33.404	14.018	1.00	26.47	APEP
ATOM	230	N	ASN	31	31.273	32.376	15.866	1.00	22.04	APEP
ATOM	231	CA	ASN	31	32.407	31.469	16.030	1.00	21.43	APEP
ATOM	232	CB	ASN	31	33.061	31.623	17.413	1.00	21.58	APEP
ATOM	233	CG	ASN	31	33.840	32.911	17.564	1.00	23.13	APEP
ATOM	234	OD1	ASN	31	34.581	33.319	16.672	1.00	23.71	APEP
ATOM	235	ND2	ASN	31	33.680	33.558	18.713	1.00	25.47	APEP
ATOM	236	C	ASN	31	31.817	30.071	15.944	1.00	19.60	APEP
ATOM	237	O	ASN	31	31.743	29.365	16.948	1.00	18.51	APEP
ATOM	238	N	CYS	32	31.384	29.667	14.756	1.00	18.76	APEP
ATOM	239	CA	CYS	32	30.779	28.348	14.605	1.00	18.03	APEP
ATOM	240	C	CYS	32	31.690	27.310	13.975	1.00	17.09	APEP
ATOM	241	O	CYS	32	31.234	26.464	13.207	1.00	13.04	APEP
ATOM	242	CB	CYS	32	29.493	28.456	13.792	1.00	17.35	APEP
ATOM	243	SG	CYS	32	28.253	29.528	14.570	1.00	16.28	APEP
ATOM	244	N	GLY	33	32.974	27.379	14.311	1.00	19.59	APEP
ATOM	245	CA	GLY	33	33.942	26.433	13.786	1.00	21.31	APEP
ATOM	246	C	GLY	33	33.914	26.269	12.278	1.00	22.56	APEP
ATOM	247	O	GLY	33	33.985	27.250	11.532	1.00	22.89	APEP
ATOM	248	N	ASN	34	33.812	25.021	11.830	1.00	22.35	APEP
ATOM	249	CA	ASN	34	33.787	24.724	10.409	1.00	23.03	APEP

ATOM	250	CB	ASN	34	34.531	23.410	10.136	1.00	26.79	APEP
ATOM	251	CG	ASN	34	33.754	22.187	10.581	1.00	31.53	APEP
ATOM	252	OD1	ASN	34	33.028	22.221	11.579	1.00	35.39	APEP
ATOM	253	ND2	ASN	34	33.908	21.088	9.840	1.00	32.88	APEP
ATOM	254	C	ASN	34	32.377	24.682	9.821	1.00	22.38	APEP
ATOM	255	O	ASN	34	32.193	24.351	8.647	1.00	21.38	APEP
ATOM	256	N	LYS	35	31.377	25.029	10.629	1.00	19.97	APEP
ATOM	257	CA	LYS	35	30.007	25.053	10.133	1.00	17.88	APEP
ATOM	258	CB	LYS	35	29.011	25.166	11.289	1.00	17.85	APEP
ATOM	259	CG	LYS	35	29.323	24.277	12.482	1.00	19.14	APEP
ATOM	260	CD	LYS	35	28.050	23.847	13.179	1.00	18.82	APEP
ATOM	261	CE	LYS	35	28.196	23.884	14.689	1.00	18.39	APEP
ATOM	262	NZ	LYS	35	29.499	23.329	15.115	1.00	18.61	APEP
ATOM	263	C	LYS	35	29.879	26.281	9.235	1.00	16.90	APEP
ATOM	264	O	LYS	35	30.557	27.284	9.453	1.00	16.79	APEP
ATOM	265	N	VAL	36	29.029	26.202	8.218	1.00	16.21	APEP
ATOM	266	CA	VAL	36	28.831	27.342	7.330	1.00	15.62	APEP
ATOM	267	CB	VAL	36	28.560	26.916	5.872	1.00	15.89	APEP
ATOM	268	CG1	VAL	36	28.474	28.150	4.990	1.00	14.85	APEP
ATOM	269	CG2	VAL	36	29.663	26.000	5.374	1.00	17.84	APEP
ATOM	270	C	VAL	36	27.636	28.149	7.820	1.00	13.37	APEP
ATOM	271	O	VAL	36	26.530	27.631	7.949	1.00	11.50	APEP
ATOM	272	N	VAL	37	27.882	29.422	8.095	1.00	13.37	APEP
ATOM	273	CA	VAL	37	26.857	30.337	8.573	1.00	15.79	APEP
ATOM	274	CB	VAL	37	27.506	31.450	9.424	1.00	16.40	APEP
ATOM	275	CG1	VAL	37	26.487	32.521	9.765	1.00	16.09	APEP
ATOM	276	CG2	VAL	37	28.096	30.847	10.681	1.00	13.21	APEP
ATOM	277	C	VAL	37	26.067	30.971	7.422	1.00	16.67	APEP
ATOM	278	O	VAL	37	26.557	31.873	6.738	1.00	18.09	APEP
ATOM	279	N	VAL	38	24.843	30.492	7.211	1.00	16.92	APEP
ATOM	280	CA	VAL	38	23.991	31.020	6.149	1.00	17.73	APEP
ATOM	281	CB	VAL	38	22.662	30.229	6.051	1.00	15.03	APEP
ATOM	282	CG1	VAL	38	21.770	30.820	4.976	1.00	15.83	APEP
ATOM	283	CG2	VAL	38	22.953	28.778	5.740	1.00	17.06	APEP
ATOM	284	C	VAL	38	23.704	32.480	6.486	1.00	17.90	APEP
ATOM	285	O	VAL	38	23.852	33.372	5.645	1.00	18.01	APEP
ATOM	286	N	SER	39	23.305	32.713	7.731	1.00	15.41	APEP
ATOM	287	CA	SER	39	23.019	34.052	8.214	1.00	14.21	APEP
ATOM	288	CB	SER	39	21.857	34.674	7.438	1.00	14.70	APEP
ATOM	289	OG	SER	39	20.721	33.837	7.467	1.00	14.28	APEP
ATOM	290	C	SER	39	22.679	34.006	9.700	1.00	14.75	APEP
ATOM	291	O	SER	39	22.636	32.936	10.308	1.00	12.05	APEP
ATOM	292	N	TYR	40	22.444	35.179	10.278	1.00	14.22	APEP
ATOM	293	CA	TYR	40	22.111	35.272	11.686	1.00	14.10	APEP
ATOM	294	CB	TYR	40	23.397	35.179	12.530	1.00	15.42	APEP
ATOM	295	CG	TYR	40	24.239	36.438	12.583	1.00	14.41	APEP
ATOM	296	CD1	TYR	40	23.921	37.472	13.464	1.00	15.34	APEP
ATOM	297	CE1	TYR	40	24.711	38.605	13.563	1.00	16.41	APEP
ATOM	298	CD2	TYR	40	25.375	36.575	11.790	1.00	14.36	APEP
ATOM	299	CE2	TYR	40	26.179	37.712	11.879	1.00	17.60	APEP
ATOM	300	CZ	TYR	40	25.842	38.723	12.771	1.00	18.38	APEP
ATOM	301	OH	TYR	40	26.639	39.841	12.896	1.00	19.23	APEP
ATOM	302	C	TYR	40	21.360	36.569	11.969	1.00	13.71	APEP
ATOM	303	O	TYR	40	21.456	37.526	11.201	1.00	13.53	APEP
ATOM	304	N	GLY	41	20.602	36.590	13.061	1.00	12.13	APEP
ATOM	305	CA	GLY	41	19.857	37.783	13.418	1.00	13.27	APEP
ATOM	306	C	GLY	41	18.381	37.656	13.102	1.00	13.46	APEP

ATOM	307	O	GLY	41	17.968	36.726	12.419	1.00	14.55	APEP
ATOM	308	N	LEU	42	17.586	38.601	13.590	1.00	12.94	APEP
ATOM	309	CA	LEU	42	16.150	38.581	13.365	1.00	12.38	APEP
ATOM	310	CB	LEU	42	15.421	38.302	14.676	1.00	11.85	APEP
ATOM	311	CG	LEU	42	15.462	36.858	15.170	1.00	9.57	APEP
ATOM	312	CD1	LEU	42	15.279	36.828	16.682	1.00	10.07	APEP
ATOM	313	CD2	LEU	42	14.374	36.063	14.475	1.00	9.98	APEP
ATOM	314	C	LEU	42	15.651	39.895	12.791	1.00	12.90	APEP
ATOM	315	O	LEU	42	16.066	40.968	13.223	1.00	13.81	APEP
ATOM	316	N	THR	43	14.758	39.808	11.816	1.00	12.41	APEP
ATOM	317	CA	THR	43	14.200	41.006	11.210	1.00	13.32	APEP
ATOM	318	CB	THR	43	13.412	40.693	9.919	1.00	11.63	APEP
ATOM	319	OG1	THR	43	12.195	40.028	10.254	1.00	12.20	APEP
ATOM	320	CG2	THR	43	14.222	39.804	8.994	1.00	11.85	APEP
ATOM	321	C	THR	43	13.249	41.637	12.208	1.00	13.30	APEP
ATOM	322	O	THR	43	12.801	40.990	13.161	1.00	12.67	APEP
ATOM	323	N	LYS	44	12.939	42.904	11.977	1.00	14.11	APEP
ATOM	324	CA	LYS	44	12.050	43.640	12.851	1.00	14.99	APEP
ATOM	325	CB	LYS	44	11.975	45.100	12.379	1.00	16.22	APEP
ATOM	326	CG	LYS	44	10.594	45.667	12.152	1.00	18.80	APEP
ATOM	327	CD	LYS	44	10.567	47.157	12.489	1.00	19.36	APEP
ATOM	328	CE	LYS	44	9.655	47.915	11.552	1.00	21.90	APEP
ATOM	329	NZ	LYS	44	10.430	48.714	10.570	1.00	20.87	APEP
ATOM	330	C	LYS	44	10.672	42.985	12.923	1.00	13.76	APEP
ATOM	331	O	LYS	44	10.083	42.910	13.999	1.00	14.04	APEP
ATOM	332	N	GLN	45	10.162	42.487	11.798	1.00	12.41	APEP
ATOM	333	CA	GLN	45	8.849	41.839	11.806	1.00	11.51	APEP
ATOM	334	CB	GLN	45	8.334	41.646	10.370	1.00	10.79	APEP
ATOM	335	CG	GLN	45	7.063	40.816	10.246	1.00	10.70	APEP
ATOM	336	CD	GLN	45	5.812	41.538	10.743	1.00	12.43	APEP
ATOM	337	OE1	GLN	45	5.696	42.763	10.650	1.00	12.72	APEP
ATOM	338	NE2	GLN	45	4.869	40.772	11.274	1.00	11.44	APEP
ATOM	339	C	GLN	45	8.917	40.496	12.548	1.00	10.87	APEP
ATOM	340	O	GLN	45	7.987	40.123	13.267	1.00	9.48	APEP
ATOM	341	N	GLU	46	10.024	39.779	12.382	1.00	9.10	APEP
ATOM	342	CA	GLU	46	10.207	38.496	13.059	1.00	9.69	APEP
ATOM	343	CB	GLU	46	11.511	37.845	12.610	1.00	8.84	APEP
ATOM	344	CG	GLU	46	11.366	36.916	11.407	1.00	9.10	APEP
ATOM	345	CD	GLU	46	12.710	36.534	10.806	1.00	9.37	APEP
ATOM	346	OE1	GLU	46	13.723	37.158	11.173	1.00	7.41	APEP
ATOM	347	OE2	GLU	46	12.755	35.607	9.966	1.00	10.21	APEP
ATOM	348	C	GLU	46	10.217	38.666	14.582	1.00	10.14	APEP
ATOM	349	O	GLU	46	9.708	37.817	15.310	1.00	10.51	APEP
ATOM	350	N	LYS	47	10.807	39.761	15.057	1.00	10.08	APEP
ATOM	351	CA	LYS	47	10.865	40.042	16.486	1.00	9.91	APEP
ATOM	352	CB	LYS	47	11.675	41.318	16.749	1.00	9.24	APEP
ATOM	353	CG	LYS	47	13.167	41.191	16.459	1.00	7.94	APEP
ATOM	354	CD	LYS	47	13.906	42.509	16.710	1.00	9.13	APEP
ATOM	355	CE	LYS	47	15.411	42.361	16.498	1.00	11.45	APEP
ATOM	356	NZ	LYS	47	16.127	43.675	16.431	1.00	11.96	APEP
ATOM	357	C	LYS	47	9.438	40.229	16.984	1.00	10.20	APEP
ATOM	358	O	LYS	47	9.027	39.626	17.969	1.00	10.41	APEP
ATOM	359	N	GLN	48	8.689	41.065	16.275	1.00	11.41	APEP
ATOM	360	CA	GLN	48	7.299	41.366	16.602	1.00	11.75	APEP
ATOM	361	CB	GLN	48	6.759	42.409	15.624	1.00	11.16	APEP
ATOM	362	CG	GLN	48	5.254	42.607	15.669	1.00	12.11	APEP
ATOM	363	CD	GLN	48	4.767	43.515	14.556	1.00	12.60	APEP

ATOM	364	OE1	GLN	48	5.301	44.606	14.359	1.00	10.04	APEP
ATOM	365	NE2	GLN	48	3.758	43.065	13.816	1.00	11.92	APEP
ATOM	366	C	GLN	48	6.420	40.123	16.563	1.00	12.69	APEP
ATOM	367	O	GLN	48	5.488	39.993	17.353	1.00	13.53	APEP
ATOM	368	N	ASP	49	6.716	39.219	15.633	1.00	12.63	APEP
ATOM	369	CA	ASP	49	5.964	37.977	15.487	1.00	11.04	APEP
ATOM	370	CB	ASP	49	6.290	37.322	14.144	1.00	14.99	APEP
ATOM	371	CG	ASP	49	5.578	37.990	12.981	1.00	17.72	APEP
ATOM	372	OD1	ASP	49	4.518	38.620	13.200	1.00	18.74	APEP
ATOM	373	OD2	ASP	49	6.082	37.878	11.844	1.00	19.80	APEP
ATOM	374	C	ASP	49	6.285	36.998	16.615	1.00	9.65	APEP
ATOM	375	O	ASP	49	5.433	36.211	17.020	1.00	9.33	APEP
ATOM	376	N	ILE	50	7.519	37.034	17.107	1.00	8.25	APEP
ATOM	377	CA	ILE	50	7.916	36.152	18.203	1.00	8.01	APEP
ATOM	378	CB	ILE	50	9.454	36.132	18.387	1.00	7.72	APEP
ATOM	379	CG2	ILE	50	9.823	35.416	19.693	1.00	7.19	APEP
ATOM	380	CG1	ILE	50	10.103	35.410	17.203	1.00	6.44	APEP
ATOM	381	CD1	ILE	50	11.582	35.687	17.041	1.00	4.97	APEP
ATOM	382	C	ILE	50	7.256	36.621	19.499	1.00	8.14	APEP
ATOM	383	O	ILE	50	6.805	35.808	20.303	1.00	7.29	APEP
ATOM	384	N	LEU	51	7.191	37.938	19.679	1.00	8.57	APEP
ATOM	385	CA	LEU	51	6.571	38.529	20.854	1.00	9.76	APEP
ATOM	386	CB	LEU	51	6.733	40.055	20.836	1.00	9.57	APEP
ATOM	387	CG	LEU	51	6.509	40.844	22.139	1.00	12.08	APEP
ATOM	388	CD1	LEU	51	7.509	40.401	23.216	1.00	9.69	APEP
ATOM	389	CD2	LEU	51	6.659	42.333	21.861	1.00	10.85	APEP
ATOM	390	C	LEU	51	5.091	38.172	20.863	1.00	10.95	APEP
ATOM	391	O	LEU	51	4.571	37.664	21.861	1.00	12.16	APEP
ATOM	392	N	LYS	52	4.423	38.427	19.739	1.00	10.41	APEP
ATOM	393	CA	LYS	52	2.994	38.156	19.601	1.00	10.29	APEP
ATOM	394	CB	LYS	52	2.520	38.535	18.196	1.00	10.94	APEP
ATOM	395	CG	LYS	52	1.066	38.956	18.132	1.00	14.35	APEP
ATOM	396	CD	LYS	52	0.258	38.029	17.236	1.00	16.34	APEP
ATOM	397	CE	LYS	52	-0.870	38.780	16.543	1.00	17.57	APEP
ATOM	398	NZ	LYS	52	-2.107	38.817	17.374	1.00	17.77	APEP
ATOM	399	C	LYS	52	2.627	36.709	19.893	1.00	9.70	APEP
ATOM	400	O	LYS	52	1.553	36.432	20.419	1.00	9.63	APEP
ATOM	401	N	GLU	53	3.508	35.780	19.540	1.00	10.85	APEP
ATOM	402	CA	GLU	53	3.249	34.366	19.799	1.00	11.95	APEP
ATOM	403	CB	GLU	53	4.261	33.491	19.057	1.00	13.57	APEP
ATOM	404	CG	GLU	53	3.957	31.996	19.089	1.00	15.51	APEP
ATOM	405	CD	GLU	53	2.525	31.651	18.695	1.00	20.29	APEP
ATOM	406	OE1	GLU	53	1.876	32.439	17.971	1.00	21.72	APEP
ATOM	407	OE2	GLU	53	2.044	30.577	19.111	1.00	21.86	APEP
ATOM	408	C	GLU	53	3.362	34.120	21.294	1.00	12.09	APEP
ATOM	409	O	GLU	53	2.568	33.382	21.878	1.00	11.99	APEP
ATOM	410	N	HIS	54	4.357	34.750	21.910	1.00	12.11	APEP
ATOM	411	CA	HIS	54	4.580	34.610	23.340	1.00	11.34	APEP
ATOM	412	CB	HIS	54	5.829	35.399	23.769	1.00	9.29	APEP
ATOM	413	CG	HIS	54	7.089	34.584	23.817	1.00	7.76	APEP
ATOM	414	CD2	HIS	54	7.695	33.933	24.840	1.00	9.61	APEP
ATOM	415	ND1	HIS	54	7.895	34.394	22.716	1.00	6.60	APEP
ATOM	416	CE1	HIS	54	8.941	33.663	23.056	1.00	5.37	APEP
ATOM	417	NE2	HIS	54	8.844	33.370	24.340	1.00	7.30	APEP
ATOM	418	C	HIS	54	3.365	35.143	24.092	1.00	10.89	APEP
ATOM	419	O	HIS	54	2.844	34.492	24.983	1.00	10.22	APEP
ATOM	420	N	ASN	55	2.913	36.331	23.703	1.00	12.43	APEP

ATOM	421	CA	ASN	55	1.784	36.982	24.356	1.00	13.65	APEP
ATOM	422	CB	ASN	55	1.791	38.473	23.991	1.00	12.77	APEP
ATOM	423	CG	ASN	55	2.950	39.232	24.655	1.00	12.31	APEP
ATOM	424	OD1	ASN	55	3.396	38.871	25.747	1.00	7.53	APEP
ATOM	425	ND2	ASN	55	3.436	40.280	23.993	1.00	9.40	APEP
ATOM	426	C	ASN	55	0.413	36.347	24.097	1.00	13.82	APEP
ATOM	427	O	ASN	55	-0.457	36.355	24.973	1.00	13.31	APEP
ATOM	428	N	ASP	56	0.221	35.795	22.907	1.00	12.69	APEP
ATOM	429	CA	ASP	56	-1.036	35.134	22.572	1.00	12.59	APEP
ATOM	430	CB	ASP	56	-1.049	34.675	21.111	1.00	12.80	APEP
ATOM	431	CG	ASP	56	-1.359	35.787	20.143	1.00	13.37	APEP
ATOM	432	OD1	ASP	56	-1.902	36.825	20.565	1.00	13.48	APEP
ATOM	433	OD2	ASP	56	-1.059	35.615	18.945	1.00	15.39	APEP
ATOM	434	C	ASP	56	-1.153	33.899	23.450	1.00	10.48	APEP
ATOM	435	O	ASP	56	-2.224	33.577	23.953	1.00	10.14	APEP
ATOM	436	N	PHE	57	-0.046	33.191	23.604	1.00	9.15	APEP
ATOM	437	CA	PHE	57	-0.047	31.989	24.418	1.00	11.09	APEP
ATOM	438	CB	PHE	57	1.272	31.227	24.252	1.00	12.68	APEP
ATOM	439	CG	PHE	57	1.261	29.863	24.884	1.00	11.85	APEP
ATOM	440	CD1	PHE	57	0.346	28.899	24.471	1.00	12.69	APEP
ATOM	441	CD2	PHE	57	2.150	29.549	25.903	1.00	12.28	APEP
ATOM	442	CE1	PHE	57	0.316	27.642	25.067	1.00	11.80	APEP
ATOM	443	CE2	PHE	57	2.132	28.296	26.508	1.00	12.07	APEP
ATOM	444	CZ	PHE	57	1.216	27.342	26.092	1.00	12.36	APEP
ATOM	445	C	PHE	57	-0.267	32.346	25.890	1.00	10.66	APEP
ATOM	446	O	PHE	57	-1.012	31.664	26.597	1.00	11.28	APEP
ATOM	447	N	ARG	58	0.360	33.423	26.349	1.00	8.83	APEP
ATOM	448	CA	ARG	58	0.204	33.830	27.738	1.00	10.25	APEP
ATOM	449	CB	ARG	58	1.107	35.024	28.057	1.00	7.50	APEP
ATOM	450	CG	ARG	58	2.483	34.615	28.530	1.00	7.56	APEP
ATOM	451	CD	ARG	58	3.478	35.755	28.446	1.00	7.29	APEP
ATOM	452	NE	ARG	58	3.391	36.649	29.601	1.00	8.58	APEP
ATOM	453	CZ	ARG	58	4.025	36.450	30.750	1.00	7.65	APEP
ATOM	454	NH1	ARG	58	4.797	35.388	30.908	1.00	8.61	APEP
ATOM	455	NH2	ARG	58	3.892	37.318	31.738	1.00	9.84	APEP
ATOM	456	C	ARG	58	-1.246	34.170	28.032	1.00	9.76	APEP
ATOM	457	O	ARG	58	-1.793	33.733	29.042	1.00	11.57	APEP
ATOM	458	N	GLN	59	-1.874	34.932	27.141	1.00	10.78	APEP
ATOM	459	CA	GLN	59	-3.270	35.321	27.314	1.00	9.25	APEP
ATOM	460	CB	GLN	59	-3.621	36.464	26.368	1.00	11.30	APEP
ATOM	461	CG	GLN	59	-3.388	37.870	26.937	1.00	14.86	APEP
ATOM	462	CD	GLN	59	-3.254	37.924	28.457	1.00	15.40	APEP
ATOM	463	OE1	GLN	59	-2.306	38.508	28.976	1.00	20.39	APEP
ATOM	464	NE2	GLN	59	-4.203	37.328	29.171	1.00	16.19	APEP
ATOM	465	C	GLN	59	-4.233	34.156	27.107	1.00	8.85	APEP
ATOM	466	O	GLN	59	-5.275	34.084	27.753	1.00	8.65	APEP
ATOM	467	N	LYS	60	-3.900	33.240	26.209	1.00	9.28	APEP
ATOM	468	CA	LYS	60	-4.765	32.084	25.999	1.00	9.29	APEP
ATOM	469	CB	LYS	60	-4.190	31.170	24.919	1.00	11.77	APEP
ATOM	470	CG	LYS	60	-5.097	29.999	24.555	1.00	12.61	APEP
ATOM	471	CD	LYS	60	-4.357	28.678	24.660	1.00	12.86	APEP
ATOM	472	CE	LYS	60	-3.849	28.205	23.310	1.00	10.33	APEP
ATOM	473	NZ	LYS	60	-4.535	26.970	22.830	1.00	12.74	APEP
ATOM	474	C	LYS	60	-4.840	31.329	27.320	1.00	9.51	APEP
ATOM	475	O	LYS	60	-5.922	31.017	27.816	1.00	7.72	APEP
ATOM	476	N	ILE	61	-3.671	31.049	27.887	1.00	9.04	APEP
ATOM	477	CA	ILE	61	-3.570	30.348	29.161	1.00	9.87	APEP

ATOM	478	CB	ILE	61	-2.075	30.101	29.536	1.00	10.78	APEP
ATOM	479	CG2	ILE	61	-1.970	29.598	30.973	1.00	10.13	APEP
ATOM	480	CG1	ILE	61	-1.428	29.152	28.507	1.00	9.94	APEP
ATOM	481	CD1	ILE	61	-1.212	27.720	28.980	1.00	10.64	APEP
ATOM	482	C	ILE	61	-4.254	31.141	30.283	1.00	9.04	APEP
ATOM	483	O	ILE	61	-4.980	30.571	31.092	1.00	9.20	APEP
ATOM	484	N	ALA	62	-4.041	32.454	30.314	1.00	8.91	APEP
ATOM	485	CA	ALA	62	-4.628	33.308	31.350	1.00	9.06	APEP
ATOM	486	CB	ALA	62	-4.090	34.729	31.209	1.00	5.84	APEP
ATOM	487	C	ALA	62	-6.165	33.327	31.363	1.00	11.19	APEP
ATOM	488	O	ALA	62	-6.794	33.581	32.397	1.00	12.79	APEP
ATOM	489	N	ARG	63	-6.769	33.053	30.214	1.00	12.40	APEP
ATOM	490	CA	ARG	63	-8.219	33.050	30.096	1.00	10.93	APEP
ATOM	491	CB	ARG	63	-8.618	33.627	28.736	1.00	10.77	APEP
ATOM	492	CG	ARG	63	-8.043	35.012	28.505	1.00	12.79	APEP
ATOM	493	CD	ARG	63	-8.608	35.684	27.278	1.00	15.66	APEP
ATOM	494	NE	ARG	63	-7.868	36.904	26.968	1.00	17.96	APEP
ATOM	495	CZ	ARG	63	-7.346	37.179	25.777	1.00	20.00	APEP
ATOM	496	NH1	ARG	63	-7.483	36.321	24.772	1.00	19.36	APEP
ATOM	497	NH2	ARG	63	-6.679	38.313	25.590	1.00	22.72	APEP
ATOM	498	C	ARG	63	-8.827	31.661	30.285	1.00	10.92	APEP
ATOM	499	O	ARG	63	-10.036	31.489	30.179	1.00	12.37	APEP
ATOM	500	N	GLY	64	-7.986	30.677	30.575	1.00	12.22	APEP
ATOM	501	CA	GLY	64	-8.475	29.325	30.780	1.00	11.71	APEP
ATOM	502	C	GLY	64	-8.985	28.685	29.509	1.00	13.26	APEP
ATOM	503	O	GLY	64	-9.950	27.911	29.540	1.00	14.03	APEP
ATOM	504	N	LEU	65	-8.331	28.998	28.391	1.00	11.24	APEP
ATOM	505	CA	LEU	65	-8.711	28.463	27.095	1.00	10.84	APEP
ATOM	506	CB	LEU	65	-8.747	29.581	26.044	1.00	10.48	APEP
ATOM	507	CG	LEU	65	-9.602	30.803	26.396	1.00	8.01	APEP
ATOM	508	CD1	LEU	65	-9.278	31.946	25.470	1.00	13.03	APEP
ATOM	509	CD2	LEU	65	-11.074	30.450	26.291	1.00	10.77	APEP
ATOM	510	C	LEU	65	-7.764	27.361	26.644	1.00	12.39	APEP
ATOM	511	O	LEU	65	-7.998	26.719	25.625	1.00	12.90	APEP
ATOM	512	N	GLU	66	-6.686	27.147	27.387	1.00	11.27	APEP
ATOM	513	CA	GLU	66	-5.754	26.094	27.023	1.00	12.09	APEP
ATOM	514	CB	GLU	66	-4.365	26.361	27.610	1.00	11.39	APEP
ATOM	515	CG	GLU	66	-3.327	25.308	27.245	1.00	12.91	APEP
ATOM	516	CD	GLU	66	-3.362	24.941	25.774	1.00	13.85	APEP
ATOM	517	OE1	GLU	66	-2.689	25.629	24.988	1.00	18.33	APEP
ATOM	518	OE2	GLU	66	-4.054	23.971	25.401	1.00	12.27	APEP
ATOM	519	C	GLU	66	-6.323	24.799	27.575	1.00	12.38	APEP
ATOM	520	O	GLU	66	-6.214	24.512	28.764	1.00	12.85	APEP
ATOM	521	N	THR	67	-6.943	24.022	26.696	1.00	13.75	APEP
ATOM	522	CA	THR	67	-7.553	22.769	27.091	1.00	13.16	APEP
ATOM	523	CB	THR	67	-8.562	22.295	26.018	1.00	13.92	APEP
ATOM	524	OG1	THR	67	-7.858	21.894	24.830	1.00	14.81	APEP
ATOM	525	CG2	THR	67	-9.524	23.413	25.671	1.00	11.42	APEP
ATOM	526	C	THR	67	-6.548	21.652	27.368	1.00	13.63	APEP
ATOM	527	O	THR	67	-6.875	20.682	28.049	1.00	14.84	APEP
ATOM	528	N	ARG	68	-5.326	21.793	26.861	1.00	13.12	APEP
ATOM	529	CA	ARG	68	-4.301	20.770	27.042	1.00	11.47	APEP
ATOM	530	CB	ARG	68	-3.222	20.914	25.967	1.00	13.83	APEP
ATOM	531	CG	ARG	68	-3.715	20.741	24.538	1.00	13.10	APEP
ATOM	532	CD	ARG	68	-2.626	21.128	23.542	1.00	13.89	APEP
ATOM	533	NE	ARG	68	-2.317	22.556	23.590	1.00	12.94	APEP
ATOM	534	CZ	ARG	68	-1.244	23.111	23.033	1.00	11.20	APEP

ATOM	535	NH1	ARG	68	-0.372	22.351	22.383	1.00	10.56	APEP
ATOM	536	NH2	ARG	68	-1.042	24.420	23.135	1.00	5.87	APEP
ATOM	537	C	ARG	68	-3.631	20.746	28.415	1.00	11.74	APEP
ATOM	538	O	ARG	68	-3.420	21.789	29.032	1.00	10.94	APEP
ATOM	539	N	GLY	69	-3.295	19.536	28.867	1.00	11.61	APEP
ATOM	540	CA	GLY	69	-2.641	19.334	30.147	1.00	14.23	APEP
ATOM	541	C	GLY	69	-2.565	17.857	30.518	1.00	16.11	APEP
ATOM	542	O	GLY	69	-2.998	17.001	29.747	1.00	16.74	APEP
ATOM	543	N	ASN	70	-2.006	17.551	31.687	1.00	16.09	APEP
ATOM	544	CA	ASN	70	-1.896	16.172	32.156	1.00	17.23	APEP
ATOM	545	CB	ASN	70	-0.439	15.704	32.132	1.00	18.05	APEP
ATOM	546	CG	ASN	70	-0.310	14.203	32.291	1.00	20.68	APEP
ATOM	547	OD1	ASN	70	-1.204	13.452	31.894	1.00	20.26	APEP
ATOM	548	ND2	ASN	70	0.806	13.752	32.874	1.00	20.67	APEP
ATOM	549	C	ASN	70	-2.452	16.025	33.578	1.00	16.86	APEP
ATOM	550	O	ASN	70	-1.717	15.739	34.523	1.00	15.70	APEP
ATOM	551	N	PRO	71	-3.770	16.204	33.738	1.00	16.37	APEP
ATOM	552	CD	PRO	71	-4.467	16.046	35.026	1.00	16.71	APEP
ATOM	553	CA	PRO	71	-4.713	16.522	32.663	1.00	16.24	APEP
ATOM	554	CB	PRO	71	-5.962	15.777	33.086	1.00	16.30	APEP
ATOM	555	CG	PRO	71	-5.928	15.906	34.614	1.00	16.81	APEP
ATOM	556	C	PRO	71	-4.999	18.012	32.491	1.00	16.23	APEP
ATOM	557	O	PRO	71	-4.638	18.837	33.338	1.00	16.06	APEP
ATOM	558	N	GLY	72	-5.666	18.342	31.392	1.00	13.86	APEP
ATOM	559	CA	GLY	72	-6.042	19.720	31.143	1.00	14.67	APEP
ATOM	560	C	GLY	72	-7.437	19.902	31.716	1.00	14.79	APEP
ATOM	561	O	GLY	72	-8.030	18.935	32.192	1.00	16.06	APEP
ATOM	562	N	PRO	73	-8.000	21.115	31.695	1.00	13.41	APEP
ATOM	563	CD	PRO	73	-9.343	21.354	32.253	1.00	13.38	APEP
ATOM	564	CA	PRO	73	-7.412	22.347	31.164	1.00	14.44	APEP
ATOM	565	CB	PRO	73	-8.621	23.250	30.976	1.00	13.74	APEP
ATOM	566	CG	PRO	73	-9.519	22.850	32.113	1.00	12.91	APEP
ATOM	567	C	PRO	73	-6.412	22.977	32.129	1.00	13.52	APEP
ATOM	568	O	PRO	73	-6.271	22.537	33.268	1.00	13.30	APEP
ATOM	569	N	GLN	74	-5.713	24.004	31.658	1.00	12.54	APEP
ATOM	570	CA	GLN	74	-4.782	24.723	32.506	1.00	11.28	APEP
ATOM	571	CB	GLN	74	-3.708	25.433	31.672	1.00	10.31	APEP
ATOM	572	CG	GLN	74	-2.658	24.505	31.043	1.00	9.27	APEP
ATOM	573	CD	GLN	74	-2.070	23.484	32.024	1.00	12.07	APEP
ATOM	574	OE1	GLN	74	-1.558	23.838	33.087	1.00	10.98	APEP
ATOM	575	NE2	GLN	74	-2.137	22.210	31.654	1.00	12.44	APEP
ATOM	576	C	GLN	74	-5.707	25.736	33.170	1.00	11.59	APEP
ATOM	577	O	GLN	74	-6.710	26.139	32.579	1.00	12.75	APEP
ATOM	578	N	PRO	75	-5.393	26.158	34.401	1.00	10.76	APEP
ATOM	579	CD	PRO	75	-4.230	25.770	35.221	1.00	10.45	APEP
ATOM	580	CA	PRO	75	-6.254	27.128	35.092	1.00	10.89	APEP
ATOM	581	CB	PRO	75	-5.876	26.948	36.561	1.00	10.78	APEP
ATOM	582	CG	PRO	75	-4.430	26.540	36.515	1.00	9.92	APEP
ATOM	583	C	PRO	75	-6.077	28.571	34.642	1.00	10.22	APEP
ATOM	584	O	PRO	75	-5.017	28.955	34.170	1.00	11.44	APEP
ATOM	585	N	PRO	76	-7.123	29.394	34.782	1.00	11.88	APEP
ATOM	586	CD	PRO	76	-8.461	29.107	35.327	1.00	13.85	APEP
ATOM	587	CA	PRO	76	-6.968	30.791	34.365	1.00	13.97	APEP
ATOM	588	CB	PRO	76	-8.383	31.373	34.440	1.00	12.73	APEP
ATOM	589	CG	PRO	76	-9.284	30.245	34.830	1.00	14.35	APEP
ATOM	590	C	PRO	76	-6.000	31.507	35.315	1.00	14.80	APEP
ATOM	591	O	PRO	76	-5.672	30.987	36.382	1.00	14.45	APEP

ATOM	592	N	ALA	77	-5.553	32.697	34.930	1.00	15.36	APEP
ATOM	593	CA	ALA	77	-4.617	33.458	35.745	1.00	16.81	APEP
ATOM	594	CB	ALA	77	-3.264	33.531	35.050	1.00	15.29	APEP
ATOM	595	C	ALA	77	-5.111	34.864	36.034	1.00	18.21	APEP
ATOM	596	O	ALA	77	-5.946	35.414	35.315	1.00	18.77	APEP
ATOM	597	N	LYS	78	-4.578	35.447	37.095	1.00	19.69	APEP
ATOM	598	CA	LYS	78	-4.944	36.799	37.487	1.00	21.45	APEP
ATOM	599	CB	LYS	78	-5.453	36.779	38.934	1.00	19.28	APEP
ATOM	600	CG	LYS	78	-5.408	38.109	39.658	1.00	21.07	APEP
ATOM	601	CD	LYS	78	-5.939	37.969	41.078	1.00	22.93	APEP
ATOM	602	CE	LYS	78	-7.039	38.993	41.380	1.00	22.07	APEP
ATOM	603	NZ	LYS	78	-8.416	38.442	41.192	1.00	18.83	APEP
ATOM	604	C	LYS	78	-3.681	37.655	37.351	1.00	20.82	APEP
ATOM	605	O	LYS	78	-3.735	38.825	36.973	1.00	22.49	APEP
ATOM	606	N	ASN	79	-2.546	37.024	37.632	1.00	20.62	APEP
ATOM	607	CA	ASN	79	-1.225	37.650	37.596	1.00	20.96	APEP
ATOM	608	CB	ASN	79	-0.322	36.893	38.591	1.00	21.73	APEP
ATOM	609	CG	ASN	79	0.895	37.696	39.041	1.00	26.41	APEP
ATOM	610	OD1	ASN	79	1.739	37.194	39.794	1.00	27.31	APEP
ATOM	611	ND2	ASN	79	0.995	38.941	38.586	1.00	30.92	APEP
ATOM	612	C	ASN	79	-0.570	37.649	36.199	1.00	20.23	APEP
ATOM	613	O	ASN	79	0.658	37.679	36.109	1.00	20.51	APEP
ATOM	614	N	MET	80	-1.354	37.648	35.117	1.00	17.31	APEP
ATOM	615	CA	MET	80	-0.745	37.581	33.783	1.00	16.95	APEP
ATOM	616	CB	MET	80	-1.334	36.398	33.014	1.00	14.01	APEP
ATOM	617	CG	MET	80	-0.475	35.941	31.848	1.00	10.95	APEP
ATOM	618	SD	MET	80	1.001	35.032	32.360	1.00	10.66	APEP
ATOM	619	CE	MET	80	0.309	33.392	32.631	1.00	9.65	APEP
ATOM	620	C	MET	80	-0.743	38.809	32.863	1.00	17.13	APEP
ATOM	621	O	MET	80	-1.785	39.236	32.377	1.00	17.76	APEP
ATOM	622	N	LYS	81	0.450	39.343	32.602	1.00	16.19	APEP
ATOM	623	CA	LYS	81	0.621	40.509	31.734	1.00	15.79	APEP
ATOM	624	CB	LYS	81	1.360	41.626	32.480	1.00	19.28	APEP
ATOM	625	CG	LYS	81	0.485	42.479	33.376	1.00	23.64	APEP
ATOM	626	CD	LYS	81	1.283	42.976	34.581	1.00	29.37	APEP
ATOM	627	CE	LYS	81	0.652	42.551	35.914	1.00	30.79	APEP
ATOM	628	NZ	LYS	81	1.639	41.850	36.794	1.00	31.43	APEP
ATOM	629	C	LYS	81	1.428	40.135	30.489	1.00	15.20	APEP
ATOM	630	O	LYS	81	2.144	39.133	30.478	1.00	13.27	APEP
ATOM	631	N	ASN	82	1.317	40.947	29.445	1.00	14.40	APEP
ATOM	632	CA	ASN	82	2.047	40.695	28.214	1.00	14.06	APEP
ATOM	633	CB	ASN	82	1.442	41.492	27.059	1.00	15.84	APEP
ATOM	634	CG	ASN	82	0.081	40.970	26.636	1.00	19.06	APEP
ATOM	635	OD1	ASN	82	-0.837	41.746	26.366	1.00	20.37	APEP
ATOM	636	ND2	ASN	82	-0.058	39.649	26.579	1.00	20.55	APEP
ATOM	637	C	ASN	82	3.496	41.107	28.400	1.00	11.87	APEP
ATOM	638	O	ASN	82	3.800	41.968	29.226	1.00	11.67	APEP
ATOM	639	N	LEU	83	4.384	40.483	27.633	1.00	10.14	APEP
ATOM	640	CA	LEU	83	5.809	40.789	27.684	1.00	9.10	APEP
ATOM	641	CB	LEU	83	6.648	39.577	27.272	1.00	9.30	APEP
ATOM	642	CG	LEU	83	6.373	38.230	27.935	1.00	9.01	APEP
ATOM	643	CD1	LEU	83	7.077	37.119	27.178	1.00	10.20	APEP
ATOM	644	CD2	LEU	83	6.843	38.283	29.378	1.00	10.62	APEP
ATOM	645	C	LEU	83	6.104	41.919	26.718	1.00	7.69	APEP
ATOM	646	O	LEU	83	5.285	42.254	25.866	1.00	7.01	APEP
ATOM	647	N	VAL	84	7.277	42.516	26.878	1.00	7.90	APEP
ATOM	648	CA	VAL	84	7.736	43.585	26.004	1.00	9.09	APEP

ATOM	649	CB	VAL	84	7.888	44.947	26.765	1.00	9.60	APEP
ATOM	650	CG1	VAL	84	6.511	45.476	27.152	1.00	11.40	APEP
ATOM	651	CG2	VAL	84	8.753	44.788	28.003	1.00	8.85	APEP
ATOM	652	C	VAL	84	9.088	43.110	25.479	1.00	8.16	APEP
ATOM	653	O	VAL	84	9.720	42.245	26.086	1.00	7.18	APEP
ATOM	654	N	TRP	85	9.524	43.637	24.343	1.00	9.08	APEP
ATOM	655	CA	TRP	85	10.807	43.222	23.801	1.00	8.24	APEP
ATOM	656	CB	TRP	85	10.844	43.412	22.283	1.00	7.78	APEP
ATOM	657	CG	TRP	85	12.054	42.789	21.623	1.00	7.96	APEP
ATOM	658	CD2	TRP	85	12.162	41.460	21.092	1.00	6.06	APEP
ATOM	659	CE2	TRP	85	13.459	41.330	20.544	1.00	5.27	APEP
ATOM	660	CE3	TRP	85	11.290	40.366	21.023	1.00	5.63	APEP
ATOM	661	CD1	TRP	85	13.260	43.392	21.384	1.00	6.75	APEP
ATOM	662	NE1	TRP	85	14.104	42.522	20.737	1.00	5.69	APEP
ATOM	663	CZ2	TRP	85	13.905	40.153	19.935	1.00	5.15	APEP
ATOM	664	CZ3	TRP	85	11.736	39.192	20.414	1.00	3.85	APEP
ATOM	665	CH2	TRP	85	13.035	39.099	19.879	1.00	3.87	APEP
ATOM	666	C	TRP	85	11.928	44.018	24.451	1.00	9.70	APEP
ATOM	667	O	TRP	85	11.790	45.214	24.713	1.00	12.63	APEP
ATOM	668	N	ASN	86	13.036	43.340	24.722	1.00	9.17	APEP
ATOM	669	CA	ASN	86	14.191	43.980	25.340	1.00	8.03	APEP
ATOM	670	CB	ASN	86	14.399	43.407	26.748	1.00	4.83	APEP
ATOM	671	CG	ASN	86	15.484	44.121	27.505	1.00	5.77	APEP
ATOM	672	OD1	ASN	86	16.657	43.826	27.332	1.00	5.23	APEP
ATOM	673	ND2	ASN	86	15.100	45.070	28.349	1.00	6.46	APEP
ATOM	674	C	ASN	86	15.450	43.789	24.474	1.00	7.66	APEP
ATOM	675	O	ASN	86	15.885	42.667	24.215	1.00	6.17	APEP
ATOM	676	N	ASP	87	16.028	44.899	24.030	1.00	8.98	APEP
ATOM	677	CA	ASP	87	17.213	44.866	23.179	1.00	9.94	APEP
ATOM	678	CB	ASP	87	17.548	46.278	22.695	1.00	10.21	APEP
ATOM	679	CG	ASP	87	16.602	46.757	21.622	1.00	9.93	APEP
ATOM	680	OD1	ASP	87	16.065	45.902	20.901	1.00	10.59	APEP
ATOM	681	OD2	ASP	87	16.392	47.980	21.498	1.00	11.15	APEP
ATOM	682	C	ASP	87	18.445	44.249	23.827	1.00	11.13	APEP
ATOM	683	O	ASP	87	19.271	43.651	23.141	1.00	11.97	APEP
ATOM	684	N	GLU	88	18.576	44.395	25.142	1.00	9.78	APEP
ATOM	685	CA	GLU	88	19.728	43.836	25.838	1.00	10.33	APEP
ATOM	686	CB	GLU	88	19.841	44.422	27.255	1.00	12.21	APEP
ATOM	687	CG	GLU	88	21.210	44.213	27.888	1.00	9.98	APEP
ATOM	688	CD	GLU	88	21.204	44.400	29.390	1.00	9.88	APEP
ATOM	689	OE1	GLU	88	20.125	44.660	29.957	1.00	13.29	APEP
ATOM	690	OE2	GLU	88	22.282	44.289	30.010	1.00	9.90	APEP
ATOM	691	C	GLU	88	19.660	42.314	25.912	1.00	9.04	APEP
ATOM	692	O	GLU	88	20.651	41.629	25.658	1.00	8.42	APEP
ATOM	693	N	LEU	89	18.491	41.789	26.269	1.00	8.21	APEP
ATOM	694	CA	LEU	89	18.305	40.343	26.367	1.00	7.65	APEP
ATOM	695	CB	LEU	89	16.881	40.016	26.824	1.00	7.64	APEP
ATOM	696	CG	LEU	89	16.499	40.394	28.254	1.00	6.63	APEP
ATOM	697	CD1	LEU	89	15.111	39.904	28.549	1.00	5.53	APEP
ATOM	698	CD2	LEU	89	17.487	39.785	29.237	1.00	7.49	APEP
ATOM	699	C	LEU	89	18.554	39.719	24.997	1.00	8.92	APEP
ATOM	700	O	LEU	89	19.214	38.689	24.885	1.00	7.56	APEP
ATOM	701	N	ALA	90	18.010	40.357	23.964	1.00	8.77	APEP
ATOM	702	CA	ALA	90	18.162	39.902	22.588	1.00	9.89	APEP
ATOM	703	CB	ALA	90	17.406	40.830	21.654	1.00	6.21	APEP
ATOM	704	C	ALA	90	19.640	39.849	22.197	1.00	9.83	APEP
ATOM	705	O	ALA	90	20.064	38.940	21.491	1.00	10.34	APEP

ATOM	706	N	TYR	91	20.415	40.821	22.672	1.00	10.22	APEP
ATOM	707	CA	TYR	91	21.846	40.894	22.380	1.00	10.21	APEP
ATOM	708	CB	TYR	91	22.426	42.203	22.921	1.00	11.27	APEP
ATOM	709	CG	TYR	91	23.921	42.329	22.730	1.00	13.72	APEP
ATOM	710	CD1	TYR	91	24.458	42.653	21.487	1.00	14.77	APEP
ATOM	711	CE1	TYR	91	25.837	42.747	21.301	1.00	16.40	APEP
ATOM	712	CD2	TYR	91	24.802	42.104	23.788	1.00	14.30	APEP
ATOM	713	CE2	TYR	91	26.178	42.195	23.614	1.00	14.06	APEP
ATOM	714	CZ	TYR	91	26.688	42.516	22.370	1.00	18.00	APEP
ATOM	715	OH	TYR	91	28.052	42.608	22.191	1.00	18.78	APEP
ATOM	716	C	TYR	91	22.620	39.714	22.967	1.00	11.02	APEP
ATOM	717	O	TYR	91	23.411	39.077	22.279	1.00	11.79	APEP
ATOM	718	N	VAL	92	22.397	39.432	24.244	1.00	10.38	APEP
ATOM	719	CA	VAL	92	23.075	38.325	24.903	1.00	8.66	APEP
ATOM	720	CB	VAL	92	22.785	38.319	26.427	1.00	8.13	APEP
ATOM	721	CG1	VAL	92	23.488	37.142	27.095	1.00	5.04	APEP
ATOM	722	CG2	VAL	92	23.267	39.622	27.046	1.00	6.97	APEP
ATOM	723	C	VAL	92	22.634	37.002	24.286	1.00	9.57	APEP
ATOM	724	O	VAL	92	23.418	36.063	24.194	1.00	10.64	APEP
ATOM	725	N	ALA	93	21.376	36.933	23.858	1.00	9.31	APEP
ATOM	726	CA	ALA	93	20.854	35.722	23.238	1.00	9.67	APEP
ATOM	727	CB	ALA	93	19.349	35.848	23.030	1.00	8.26	APEP
ATOM	728	C	ALA	93	21.561	35.489	21.898	1.00	9.72	APEP
ATOM	729	O	ALA	93	21.954	34.366	21.581	1.00	10.89	APEP
ATOM	730	N	GLN	94	21.730	36.565	21.130	1.00	8.57	APEP
ATOM	731	CA	GLN	94	22.386	36.515	19.828	1.00	6.19	APEP
ATOM	732	CB	GLN	94	22.316	37.892	19.162	1.00	7.13	APEP
ATOM	733	CG	GLN	94	22.606	37.891	17.668	1.00	6.55	APEP
ATOM	734	CD	GLN	94	21.778	36.875	16.911	1.00	6.86	APEP
ATOM	735	OE1	GLN	94	20.551	37.018	16.775	1.00	7.69	APEP
ATOM	736	NE2	GLN	94	22.441	35.836	16.412	1.00	4.45	APEP
ATOM	737	C	GLN	94	23.843	36.082	19.946	1.00	7.41	APEP
ATOM	738	O	GLN	94	24.302	35.217	19.203	1.00	8.55	APEP
ATOM	739	N	VAL	95	24.574	36.699	20.868	1.00	7.81	APEP
ATOM	740	CA	VAL	95	25.971	36.357	21.089	1.00	6.18	APEP
ATOM	741	CB	VAL	95	26.551	37.112	22.327	1.00	8.06	APEP
ATOM	742	CG1	VAL	95	27.899	36.523	22.728	1.00	8.13	APEP
ATOM	743	CG2	VAL	95	26.716	38.583	22.011	1.00	7.34	APEP
ATOM	744	C	VAL	95	26.091	34.855	21.324	1.00	7.07	APEP
ATOM	745	O	VAL	95	26.949	34.199	20.737	1.00	3.91	APEP
ATOM	746	N	TRP	96	25.224	34.312	22.180	1.00	8.26	APEP
ATOM	747	CA	TRP	96	25.244	32.879	22.494	1.00	8.88	APEP
ATOM	748	CB	TRP	96	24.284	32.555	23.650	1.00	6.54	APEP
ATOM	749	CG	TRP	96	24.258	31.089	24.030	1.00	7.96	APEP
ATOM	750	CD2	TRP	96	25.390	30.232	24.240	1.00	7.64	APEP
ATOM	751	CE2	TRP	96	24.892	28.946	24.549	1.00	7.17	APEP
ATOM	752	CE3	TRP	96	26.778	30.426	24.197	1.00	8.39	APEP
ATOM	753	CD1	TRP	96	23.150	30.305	24.217	1.00	8.48	APEP
ATOM	754	NE1	TRP	96	23.524	29.016	24.527	1.00	5.50	APEP
ATOM	755	CZ2	TRP	96	25.734	27.859	24.812	1.00	6.91	APEP
ATOM	756	CZ3	TRP	96	27.614	29.341	24.459	1.00	8.97	APEP
ATOM	757	CH2	TRP	96	27.087	28.076	24.761	1.00	8.90	APEP
ATOM	758	C	TRP	96	24.867	32.033	21.281	1.00	8.85	APEP
ATOM	759	O	TRP	96	25.500	31.011	21.007	1.00	8.27	APEP
ATOM	760	N	ALA	97	23.827	32.453	20.566	1.00	7.57	APEP
ATOM	761	CA	ALA	97	23.390	31.721	19.381	1.00	9.88	APEP
ATOM	762	CB	ALA	97	22.182	32.415	18.742	1.00	4.36	APEP

ATOM	763	C	ALA	97	24.547	31.665	18.387	1.00	8.40	APEP
ATOM	764	O	ALA	97	24.777	30.647	17.734	1.00	8.69	APEP
ATOM	765	N	ASN	98	25.282	32.767	18.300	1.00	9.32	APEP
ATOM	766	CA	ASN	98	26.402	32.883	17.375	1.00	9.40	APEP
ATOM	767	CB	ASN	98	26.898	34.336	17.347	1.00	8.07	APEP
ATOM	768	CG	ASN	98	26.084	35.217	16.402	1.00	8.00	APEP
ATOM	769	OD1	ASN	98	25.093	34.776	15.821	1.00	11.11	APEP
ATOM	770	ND2	ASN	98	26.500	36.464	16.250	1.00	9.71	APEP
ATOM	771	C	ASN	98	27.568	31.926	17.647	1.00	9.78	APEP
ATOM	772	O	ASN	98	28.524	31.874	16.869	1.00	8.97	APEP
ATOM	773	N	GLN	99	27.492	31.160	18.733	1.00	8.27	APEP
ATOM	774	CA	GLN	99	28.556	30.212	19.051	1.00	9.27	APEP
ATOM	775	CB	GLN	99	28.774	30.120	20.572	1.00	10.68	APEP
ATOM	776	CG	GLN	99	29.117	31.452	21.241	1.00	9.08	APEP
ATOM	777	CD	GLN	99	30.119	32.266	20.444	1.00	10.60	APEP
ATOM	778	OE1	GLN	99	31.195	31.780	20.107	1.00	11.69	APEP
ATOM	779	NE2	GLN	99	29.772	33.511	20.146	1.00	12.17	APEP
ATOM	780	C	GLN	99	28.205	28.839	18.484	1.00	10.52	APEP
ATOM	781	O	GLN	99	29.049	27.942	18.426	1.00	11.67	APEP
ATOM	782	N	CYS	100	26.959	28.690	18.047	1.00	11.03	APEP
ATOM	783	CA	CYS	100	26.474	27.439	17.470	1.00	12.62	APEP
ATOM	784	C	CYS	100	26.711	26.234	18.373	1.00	14.10	APEP
ATOM	785	O	CYS	100	27.113	25.166	17.906	1.00	13.71	APEP
ATOM	786	CB	CYS	100	27.126	27.182	16.108	1.00	12.51	APEP
ATOM	787	SG	CYS	100	26.639	28.321	14.766	1.00	13.92	APEP
ATOM	788	N	GLN	101	26.457	26.411	19.667	1.00	13.78	APEP
ATOM	789	CA	GLN	101	26.615	25.337	20.640	1.00	14.58	APEP
ATOM	790	CB	GLN	101	27.656	25.723	21.696	1.00	16.78	APEP
ATOM	791	CG	GLN	101	29.106	25.506	21.269	1.00	19.68	APEP
ATOM	792	CD	GLN	101	30.097	26.125	22.239	1.00	20.61	APEP
ATOM	793	OE1	GLN	101	31.113	26.690	21.833	1.00	23.08	APEP
ATOM	794	NE2	GLN	101	29.802	26.023	23.530	1.00	24.57	APEP
ATOM	795	C	GLN	101	25.272	25.098	21.323	1.00	14.56	APEP
ATOM	796	O	GLN	101	24.987	25.716	22.347	1.00	14.71	APEP
ATOM	797	N	TYR	102	24.457	24.201	20.767	1.00	12.66	APEP
ATOM	798	CA	TYR	102	23.131	23.911	21.326	1.00	14.54	APEP
ATOM	799	CB	TYR	102	22.469	22.721	20.610	1.00	13.93	APEP
ATOM	800	CG	TYR	102	21.015	22.531	21.012	1.00	13.05	APEP
ATOM	801	CD1	TYR	102	20.033	23.418	20.574	1.00	11.54	APEP
ATOM	802	CE1	TYR	102	18.710	23.295	20.990	1.00	10.81	APEP
ATOM	803	CD2	TYR	102	20.632	21.505	21.881	1.00	13.43	APEP
ATOM	804	CE2	TYR	102	19.298	21.373	22.307	1.00	13.52	APEP
ATOM	805	CZ	TYR	102	18.348	22.276	21.853	1.00	11.37	APEP
ATOM	806	OH	TYR	102	17.031	22.154	22.242	1.00	12.72	APEP
ATOM	807	C	TYR	102	23.123	23.636	22.824	1.00	14.75	APEP
ATOM	808	O	TYR	102	23.825	22.747	23.305	1.00	14.15	APEP
ATOM	809	N	GLY	103	22.303	24.399	23.548	1.00	15.34	APEP
ATOM	810	CA	GLY	103	22.194	24.241	24.988	1.00	13.83	APEP
ATOM	811	C	GLY	103	22.174	25.586	25.698	1.00	14.82	APEP
ATOM	812	O	GLY	103	22.051	26.627	25.050	1.00	13.51	APEP
ATOM	813	N	HIS	104	22.309	25.576	27.022	1.00	13.28	APEP
ATOM	814	CA	HIS	104	22.293	26.821	27.792	1.00	13.00	APEP
ATOM	815	CB	HIS	104	21.535	26.627	29.111	1.00	14.84	APEP
ATOM	816	CG	HIS	104	20.085	26.309	28.938	1.00	17.77	APEP
ATOM	817	CD2	HIS	104	19.345	25.263	29.370	1.00	18.90	APEP
ATOM	818	ND1	HIS	104	19.224	27.125	28.236	1.00	19.74	APEP
ATOM	819	CE1	HIS	104	18.014	26.594	28.245	1.00	19.42	APEP

ATOM	820	NE2	HIS	104	18.060	25.465	28.925	1.00	19.61	APEP
ATOM	821	C	HIS	104	23.689	27.322	28.116	1.00	10.17	APEP
ATOM	822	O	HIS	104	24.573	26.532	28.403	1.00	8.70	APEP
ATOM	823	N	ASP	105	23.890	28.635	28.058	1.00	10.86	APEP
ATOM	824	CA	ASP	105	25.188	29.197	28.417	1.00	12.42	APEP
ATOM	825	CB	ASP	105	25.400	30.590	27.794	1.00	10.99	APEP
ATOM	826	CG	ASP	105	24.172	31.463	27.875	1.00	11.97	APEP
ATOM	827	OD1	ASP	105	23.054	30.914	27.966	1.00	14.08	APEP
ATOM	828	OD2	ASP	105	24.324	32.705	27.844	1.00	11.83	APEP
ATOM	829	C	ASP	105	25.200	29.274	29.949	1.00	13.37	APEP
ATOM	830	O	ASP	105	24.145	29.250	30.592	1.00	13.12	APEP
ATOM	831	N	THR	106	26.395	29.361	30.522	1.00	14.55	APEP
ATOM	832	CA	THR	106	26.573	29.385	31.971	1.00	15.70	APEP
ATOM	833	CB	THR	106	28.032	29.051	32.322	1.00	17.02	APEP
ATOM	834	OG1	THR	106	28.349	27.739	31.837	1.00	19.67	APEP
ATOM	835	CG2	THR	106	28.244	29.101	33.815	1.00	19.92	APEP
ATOM	836	C	THR	106	26.181	30.661	32.712	1.00	14.86	APEP
ATOM	837	O	THR	106	25.648	30.598	33.826	1.00	14.67	APEP
ATOM	838	N	CYS	107	26.444	31.813	32.107	1.00	12.86	APEP
ATOM	839	CA	CYS	107	26.131	33.086	32.748	1.00	11.94	APEP
ATOM	840	C	CYS	107	25.608	34.112	31.741	1.00	11.28	APEP
ATOM	841	O	CYS	107	26.354	34.594	30.886	1.00	8.75	APEP
ATOM	842	CB	CYS	107	27.389	33.618	33.451	1.00	11.80	APEP
ATOM	843	SG	CYS	107	27.155	35.045	34.567	1.00	14.81	APEP
ATOM	844	N	ARG	108	24.324	34.448	31.857	1.00	10.42	APEP
ATOM	845	CA	ARG	108	23.694	35.408	30.956	1.00	10.25	APEP
ATOM	846	CB	ARG	108	22.656	34.703	30.080	1.00	7.60	APEP
ATOM	847	CG	ARG	108	21.299	34.525	30.746	1.00	6.23	APEP
ATOM	848	CD	ARG	108	20.458	33.460	30.047	1.00	4.46	APEP
ATOM	849	NE	ARG	108	21.066	32.136	30.118	1.00	8.96	APEP
ATOM	850	CZ	ARG	108	20.688	31.192	30.971	1.00	9.87	APEP
ATOM	851	NH1	ARG	108	19.703	31.427	31.825	1.00	9.20	APEP
ATOM	852	NH2	ARG	108	21.284	30.013	30.968	1.00	9.69	APEP
ATOM	853	C	ARG	108	23.015	36.575	31.667	1.00	10.62	APEP
ATOM	854	O	ARG	108	22.465	37.454	31.011	1.00	11.81	APEP
ATOM	855	N	ASP	109	23.051	36.583	32.998	1.00	10.19	APEP
ATOM	856	CA	ASP	109	22.421	37.644	33.784	1.00	9.53	APEP
ATOM	857	CB	ASP	109	22.737	37.457	35.267	1.00	11.00	APEP
ATOM	858	CG	ASP	109	22.049	36.248	35.864	1.00	10.30	APEP
ATOM	859	OD1	ASP	109	21.137	35.704	35.213	1.00	8.82	APEP
ATOM	860	OD2	ASP	109	22.420	35.839	36.984	1.00	12.03	APEP
ATOM	861	C	ASP	109	22.827	39.051	33.368	1.00	10.45	APEP
ATOM	862	O	ASP	109	23.931	39.274	32.878	1.00	11.18	APEP
ATOM	863	N	VAL	110	21.919	40.001	33.565	1.00	10.47	APEP
ATOM	864	CA	VAL	110	22.192	41.400	33.240	1.00	11.15	APEP
ATOM	865	CB	VAL	110	21.083	42.025	32.346	1.00	8.57	APEP
ATOM	866	CG1	VAL	110	21.282	41.607	30.884	1.00	8.96	APEP
ATOM	867	CG2	VAL	110	19.711	41.600	32.840	1.00	8.45	APEP
ATOM	868	C	VAL	110	22.263	42.168	34.564	1.00	12.34	APEP
ATOM	869	O	VAL	110	22.044	41.591	35.631	1.00	11.18	APEP
ATOM	870	N	ALA	111	22.567	43.460	34.493	1.00	12.60	APEP
ATOM	871	CA	ALA	111	22.670	44.283	35.691	1.00	14.41	APEP
ATOM	872	CB	ALA	111	23.192	45.665	35.329	1.00	14.98	APEP
ATOM	873	C	ALA	111	21.351	44.412	36.445	1.00	15.05	APEP
ATOM	874	O	ALA	111	21.348	44.491	37.665	1.00	17.05	APEP
ATOM	875	N	LYS	112	20.233	44.425	35.723	1.00	15.22	APEP
ATOM	876	CA	LYS	112	18.919	44.565	36.346	1.00	14.82	APEP

ATOM	877	CB	LYS	112	17.889	44.979	35.295	1.00	17.33	APEP
ATOM	878	CG	LYS	112	16.518	45.301	35.854	1.00	18.63	APEP
ATOM	879	CD	LYS	112	15.722	46.156	34.885	1.00	20.78	APEP
ATOM	880	CE	LYS	112	14.275	46.298	35.331	1.00	22.40	APEP
ATOM	881	NZ	LYS	112	13.378	46.775	34.230	1.00	24.78	APEP
ATOM	882	C	LYS	112	18.395	43.334	37.092	1.00	14.63	APEP
ATOM	883	O	LYS	112	17.763	43.462	38.138	1.00	15.19	APEP
ATOM	884	N	TYR	113	18.652	42.145	36.565	1.00	14.03	APEP
ATOM	885	CA	TYR	113	18.155	40.941	37.211	1.00	12.90	APEP
ATOM	886	CB	TYR	113	16.627	40.866	37.062	1.00	14.34	APEP
ATOM	887	CG	TYR	113	16.094	41.275	35.701	1.00	13.96	APEP
ATOM	888	CD1	TYR	113	16.725	40.867	34.529	1.00	14.86	APEP
ATOM	889	CE1	TYR	113	16.236	41.234	33.279	1.00	15.41	APEP
ATOM	890	CD2	TYR	113	14.950	42.064	35.590	1.00	15.52	APEP
ATOM	891	CE2	TYR	113	14.447	42.439	34.345	1.00	17.15	APEP
ATOM	892	CZ	TYR	113	15.098	42.021	33.192	1.00	18.32	APEP
ATOM	893	OH	TYR	113	14.619	42.406	31.958	1.00	20.11	APEP
ATOM	894	C	TYR	113	18.761	39.659	36.658	1.00	12.75	APEP
ATOM	895	O	TYR	113	19.592	39.685	35.742	1.00	9.39	APEP
ATOM	896	N	GLN	114	18.334	38.542	37.241	1.00	11.10	APEP
ATOM	897	CA	GLN	114	18.762	37.223	36.820	1.00	11.13	APEP
ATOM	898	CB	GLN	114	18.397	36.182	37.872	1.00	13.13	APEP
ATOM	899	CG	GLN	114	19.492	35.921	38.881	1.00	17.82	APEP
ATOM	900	CD	GLN	114	19.049	34.969	39.971	1.00	21.01	APEP
ATOM	901	OE1	GLN	114	18.984	33.754	39.767	1.00	24.17	APEP
ATOM	902	NE2	GLN	114	18.735	35.517	41.140	1.00	21.13	APEP
ATOM	903	C	GLN	114	17.969	36.977	35.549	1.00	10.94	APEP
ATOM	904	O	GLN	114	16.851	37.489	35.418	1.00	10.27	APEP
ATOM	905	N	VAL	115	18.529	36.195	34.626	1.00	8.78	APEP
ATOM	906	CA	VAL	115	17.879	35.936	33.339	1.00	6.98	APEP
ATOM	907	CB	VAL	115	18.679	36.628	32.204	1.00	8.14	APEP
ATOM	908	CG1	VAL	115	18.037	36.358	30.868	1.00	9.82	APEP
ATOM	909	CG2	VAL	115	18.750	38.125	32.461	1.00	6.98	APEP
ATOM	910	C	VAL	115	17.669	34.457	32.975	1.00	6.17	APEP
ATOM	911	O	VAL	115	18.581	33.634	33.093	1.00	4.78	APEP
ATOM	912	N	GLY	116	16.449	34.142	32.540	1.00	5.97	APEP
ATOM	913	CA	GLY	116	16.105	32.788	32.139	1.00	7.14	APEP
ATOM	914	C	GLY	116	16.364	32.568	30.654	1.00	7.72	APEP
ATOM	915	O	GLY	116	16.706	33.504	29.930	1.00	6.29	APEP
ATOM	916	N	GLN	117	16.195	31.337	30.186	1.00	8.62	APEP
ATOM	917	CA	GLN	117	16.456	31.058	28.780	1.00	9.69	APEP
ATOM	918	CB	GLN	117	17.980	31.001	28.550	1.00	8.96	APEP
ATOM	919	CG	GLN	117	18.419	30.465	27.179	1.00	8.07	APEP
ATOM	920	CD	GLN	117	19.935	30.331	27.046	1.00	7.90	APEP
ATOM	921	OE1	GLN	117	20.507	29.288	27.360	1.00	10.49	APEP
ATOM	922	NE2	GLN	117	20.586	31.386	26.575	1.00	7.02	APEP
ATOM	923	C	GLN	117	15.813	29.790	28.230	1.00	9.46	APEP
ATOM	924	O	GLN	117	15.713	28.775	28.920	1.00	8.69	APEP
ATOM	925	N	ASN	118	15.372	29.876	26.978	1.00	10.04	APEP
ATOM	926	CA	ASN	118	14.762	28.759	26.253	1.00	9.76	APEP
ATOM	927	CB	ASN	118	13.280	29.037	25.950	1.00	9.04	APEP
ATOM	928	CG	ASN	118	12.357	28.735	27.127	1.00	9.64	APEP
ATOM	929	OD1	ASN	118	12.696	27.976	28.035	1.00	9.37	APEP
ATOM	930	ND2	ASN	118	11.178	29.337	27.108	1.00	8.63	APEP
ATOM	931	C	ASN	118	15.526	28.674	24.926	1.00	9.88	APEP
ATOM	932	O	ASN	118	15.847	29.707	24.342	1.00	9.07	APEP
ATOM	933	N	VAL	119	15.836	27.464	24.465	1.00	10.24	APEP

ATOM	934	CA	VAL	119	16.533	27.288	23.188	1.00	9.82	APEP
ATOM	935	CB	VAL	119	18.021	26.811	23.340	1.00	9.70	APEP
ATOM	936	CG1	VAL	119	18.764	27.684	24.349	1.00	11.24	APEP
ATOM	937	CG2	VAL	119	18.072	25.344	23.749	1.00	11.10	APEP
ATOM	938	C	VAL	119	15.784	26.247	22.379	1.00	10.42	APEP
ATOM	939	O	VAL	119	15.116	25.380	22.939	1.00	7.84	APEP
ATOM	940	N	ALA	120	15.894	26.345	21.057	1.00	11.69	APEP
ATOM	941	CA	ALA	120	15.224	25.416	20.164	1.00	10.58	APEP
ATOM	942	CB	ALA	120	13.853	25.960	19.783	1.00	9.38	APEP
ATOM	943	C	ALA	120	16.065	25.203	18.913	1.00	11.91	APEP
ATOM	944	O	ALA	120	16.749	26.114	18.447	1.00	11.05	APEP
ATOM	945	N	LEU	121	16.005	23.999	18.363	1.00	11.80	APEP
ATOM	946	CA	LEU	121	16.762	23.707	17.164	1.00	10.62	APEP
ATOM	947	CB	LEU	121	18.219	23.423	17.534	1.00	12.00	APEP
ATOM	948	CG	LEU	121	19.162	23.065	16.383	1.00	14.58	APEP
ATOM	949	CD1	LEU	121	19.914	24.310	15.937	1.00	14.77	APEP
ATOM	950	CD2	LEU	121	20.124	21.975	16.830	1.00	16.44	APEP
ATOM	951	C	LEU	121	16.190	22.521	16.395	1.00	11.28	APEP
ATOM	952	O	LEU	121	15.744	21.540	16.989	1.00	8.33	APEP
ATOM	953	N	THR	122	16.183	22.633	15.069	1.00	9.47	APEP
ATOM	954	CA	THR	122	15.723	21.551	14.203	1.00	9.80	APEP
ATOM	955	CB	THR	122	14.282	21.766	13.691	1.00	8.73	APEP
ATOM	956	OG1	THR	122	14.272	22.801	12.704	1.00	8.66	APEP
ATOM	957	CG2	THR	122	13.357	22.133	14.838	1.00	11.47	APEP
ATOM	958	C	THR	122	16.666	21.502	13.009	1.00	9.64	APEP
ATOM	959	O	THR	122	17.232	22.524	12.616	1.00	9.15	APEP
ATOM	960	N	GLY	123	16.847	20.308	12.451	1.00	9.74	APEP
ATOM	961	CA	GLY	123	17.728	20.137	11.313	1.00	8.54	APEP
ATOM	962	C	GLY	123	17.048	19.326	10.228	1.00	8.95	APEP
ATOM	963	O	GLY	123	16.199	18.482	10.514	1.00	9.03	APEP
ATOM	964	N	SER	124	17.420	19.580	8.979	1.00	7.43	APEP
ATOM	965	CA	SER	124	16.824	18.874	7.857	1.00	9.14	APEP
ATOM	966	CB	SER	124	15.584	19.642	7.393	1.00	10.09	APEP
ATOM	967	OG	SER	124	15.333	19.459	6.016	1.00	11.96	APEP
ATOM	968	C	SER	124	17.827	18.718	6.709	1.00	9.54	APEP
ATOM	969	O	SER	124	18.716	19.551	6.537	1.00	10.56	APEP
ATOM	970	N	THR	125	17.693	17.641	5.936	1.00	10.06	APEP
ATOM	971	CA	THR	125	18.591	17.415	4.812	1.00	10.19	APEP
ATOM	972	CB	THR	125	18.513	15.974	4.257	1.00	11.20	APEP
ATOM	973	OG1	THR	125	17.142	15.593	4.086	1.00	12.88	APEP
ATOM	974	CG2	THR	125	19.218	15.001	5.191	1.00	8.70	APEP
ATOM	975	C	THR	125	18.274	18.369	3.676	1.00	9.96	APEP
ATOM	976	O	THR	125	19.081	18.532	2.772	1.00	10.12	APEP
ATOM	977	N	ALA	126	17.103	18.999	3.731	1.00	10.40	APEP
ATOM	978	CA	ALA	126	16.678	19.955	2.705	1.00	11.31	APEP
ATOM	979	CB	ALA	126	15.169	19.863	2.492	1.00	11.00	APEP
ATOM	980	C	ALA	126	17.060	21.383	3.086	1.00	13.19	APEP
ATOM	981	O	ALA	126	17.116	21.735	4.271	1.00	12.55	APEP
ATOM	982	N	ALA	127	17.314	22.207	2.078	1.00	12.78	APEP
ATOM	983	CA	ALA	127	17.700	23.590	2.315	1.00	15.72	APEP
ATOM	984	CB	ALA	127	18.471	24.135	1.106	1.00	15.36	APEP
ATOM	985	C	ALA	127	16.496	24.474	2.610	1.00	17.23	APEP
ATOM	986	O	ALA	127	16.080	25.271	1.773	1.00	17.44	APEP
ATOM	987	N	LYS	128	15.941	24.324	3.810	1.00	19.74	APEP
ATOM	988	CA	LYS	128	14.790	25.110	4.251	1.00	19.25	APEP
ATOM	989	CB	LYS	128	13.481	24.387	3.917	1.00	21.28	APEP
ATOM	990	CG	LYS	128	12.930	24.721	2.527	1.00	26.82	APEP

ATOM	991	CD	LYS	128	12.083	25.993	2.549	1.00	27.74	APEP
ATOM	992	CE	LYS	128	11.582	26.365	1.152	1.00	27.56	APEP
ATOM	993	NZ	LYS	128	10.376	27.258	1.191	1.00	24.53	APEP
ATOM	994	C	LYS	128	14.918	25.311	5.760	1.00	20.56	APEP
ATOM	995	O	LYS	128	15.299	24.384	6.488	1.00	18.95	APEP
ATOM	996	N	TYR	129	14.599	26.517	6.224	1.00	19.02	APEP
ATOM	997	CA	TYR	129	14.712	26.853	7.644	1.00	18.90	APEP
ATOM	998	CB	TYR	129	15.728	27.985	7.812	1.00	17.17	APEP
ATOM	999	CG	TYR	129	17.060	27.645	7.188	1.00	15.78	APEP
ATOM	1000	CD1	TYR	129	17.319	27.934	5.847	1.00	15.45	APEP
ATOM	1001	CE1	TYR	129	18.519	27.564	5.250	1.00	13.12	APEP
ATOM	1002	CD2	TYR	129	18.043	26.984	7.918	1.00	16.01	APEP
ATOM	1003	CE2	TYR	129	19.250	26.610	7.330	1.00	16.66	APEP
ATOM	1004	CZ	TYR	129	19.479	26.900	5.994	1.00	15.93	APEP
ATOM	1005	OH	TYR	129	20.652	26.495	5.404	1.00	11.93	APEP
ATOM	1006	C	TYR	129	13.384	27.213	8.312	1.00	18.94	APEP
ATOM	1007	O	TYR	129	12.574	27.980	7.775	1.00	19.85	APEP
ATOM	1008	N	ASP	130	13.178	26.645	9.496	1.00	17.23	APEP
ATOM	1009	CA	ASP	130	11.953	26.844	10.259	1.00	16.64	APEP
ATOM	1010	CB	ASP	130	12.012	26.050	11.568	1.00	18.70	APEP
ATOM	1011	CG	ASP	130	11.399	24.677	11.446	1.00	18.76	APEP
ATOM	1012	OD1	ASP	130	11.067	24.267	10.319	1.00	17.38	APEP
ATOM	1013	OD2	ASP	130	11.253	24.005	12.489	1.00	20.96	APEP
ATOM	1014	C	ASP	130	11.615	28.285	10.588	1.00	14.91	APEP
ATOM	1015	O	ASP	130	12.489	29.111	10.831	1.00	14.08	APEP
ATOM	1016	N	ASP	131	10.317	28.557	10.584	1.00	16.25	APEP
ATOM	1017	CA	ASP	131	9.759	29.858	10.911	1.00	16.60	APEP
ATOM	1018	CB	ASP	131	8.255	29.834	10.571	1.00	19.29	APEP
ATOM	1019	CG	ASP	131	7.558	31.166	10.807	1.00	24.36	APEP
ATOM	1020	OD1	ASP	131	8.036	31.978	11.630	1.00	27.58	APEP
ATOM	1021	OD2	ASP	131	6.506	31.396	10.168	1.00	28.19	APEP
ATOM	1022	C	ASP	131	9.993	29.961	12.428	1.00	15.04	APEP
ATOM	1023	O	ASP	131	9.708	29.012	13.159	1.00	14.34	APEP
ATOM	1024	N	PRO	132	10.534	31.092	12.910	1.00	12.77	APEP
ATOM	1025	CD	PRO	132	10.950	32.276	12.139	1.00	12.42	APEP
ATOM	1026	CA	PRO	132	10.788	31.253	14.354	1.00	13.80	APEP
ATOM	1027	CB	PRO	132	11.197	32.722	14.492	1.00	13.81	APEP
ATOM	1028	CG	PRO	132	11.711	33.104	13.149	1.00	14.43	APEP
ATOM	1029	C	PRO	132	9.592	30.895	15.251	1.00	13.34	APEP
ATOM	1030	O	PRO	132	9.758	30.239	16.278	1.00	12.55	APEP
ATOM	1031	N	VAL	133	8.396	31.325	14.850	1.00	12.80	APEP
ATOM	1032	CA	VAL	133	7.170	31.054	15.591	1.00	12.13	APEP
ATOM	1033	CB	VAL	133	5.944	31.661	14.862	1.00	11.80	APEP
ATOM	1034	CG1	VAL	133	4.653	31.032	15.364	1.00	10.96	APEP
ATOM	1035	CG2	VAL	133	5.923	33.159	15.064	1.00	13.44	APEP
ATOM	1036	C	VAL	133	6.964	29.549	15.744	1.00	13.10	APEP
ATOM	1037	O	VAL	133	6.452	29.083	16.763	1.00	11.95	APEP
ATOM	1038	N	LYS	134	7.361	28.796	14.721	1.00	13.08	APEP
ATOM	1039	CA	LYS	134	7.227	27.341	14.738	1.00	14.09	APEP
ATOM	1040	CB	LYS	134	7.594	26.756	13.374	1.00	14.78	APEP
ATOM	1041	CG	LYS	134	7.716	25.238	13.367	1.00	17.92	APEP
ATOM	1042	CD	LYS	134	7.273	24.661	12.024	1.00	20.75	APEP
ATOM	1043	CE	LYS	134	7.454	23.147	11.974	1.00	21.88	APEP
ATOM	1044	NZ	LYS	134	7.979	22.704	10.646	1.00	22.20	APEP
ATOM	1045	C	LYS	134	8.125	26.734	15.805	1.00	13.26	APEP
ATOM	1046	O	LYS	134	7.775	25.732	16.437	1.00	12.31	APEP
ATOM	1047	N	LEU	135	9.289	27.343	15.990	1.00	12.25	APEP

ATOM	1048	CA	LEU	135	10.245	26.883	16.987	1.00	12.29	APEP
ATOM	1049	CB	LEU	135	11.604	27.551	16.755	1.00	11.92	APEP
ATOM	1050	CG	LEU	135	12.371	26.968	15.563	1.00	12.40	APEP
ATOM	1051	CD1	LEU	135	13.673	27.703	15.354	1.00	10.11	APEP
ATOM	1052	CD2	LEU	135	12.633	25.492	15.816	1.00	13.69	APEP
ATOM	1053	C	LEU	135	9.711	27.222	18.371	1.00	11.79	APEP
ATOM	1054	O	LEU	135	9.862	26.443	19.311	1.00	12.52	APEP
ATOM	1055	N	VAL	136	9.070	28.378	18.492	1.00	11.76	APEP
ATOM	1056	CA	VAL	136	8.507	28.805	19.773	1.00	11.55	APEP
ATOM	1057	CB	VAL	136	7.926	30.236	19.674	1.00	9.15	APEP
ATOM	1058	CG1	VAL	136	7.043	30.531	20.874	1.00	9.61	APEP
ATOM	1059	CG2	VAL	136	9.053	31.247	19.587	1.00	5.53	APEP
ATOM	1060	C	VAL	136	7.405	27.836	20.227	1.00	12.73	APEP
ATOM	1061	O	VAL	136	7.370	27.421	21.385	1.00	11.68	APEP
ATOM	1062	N	LYS	137	6.521	27.477	19.298	1.00	13.14	APEP
ATOM	1063	CA	LYS	137	5.422	26.554	19.563	1.00	12.46	APEP
ATOM	1064	CB	LYS	137	4.562	26.376	18.307	1.00	12.12	APEP
ATOM	1065	CG	LYS	137	3.866	27.659	17.847	1.00	16.24	APEP
ATOM	1066	CD	LYS	137	2.763	27.391	16.836	1.00	13.34	APEP
ATOM	1067	CE	LYS	137	1.560	28.297	17.064	1.00	16.29	APEP
ATOM	1068	NZ	LYS	137	0.433	27.565	17.706	1.00	12.20	APEP
ATOM	1069	C	LYS	137	5.939	25.198	20.031	1.00	12.60	APEP
ATOM	1070	O	LYS	137	5.183	24.406	20.589	1.00	13.30	APEP
ATOM	1071	N	MET	138	7.220	24.924	19.797	1.00	12.70	APEP
ATOM	1072	CA	MET	138	7.807	23.662	20.240	1.00	15.80	APEP
ATOM	1073	CB	MET	138	9.266	23.545	19.779	1.00	17.09	APEP
ATOM	1074	CG	MET	138	9.478	22.767	18.482	1.00	21.36	APEP
ATOM	1075	SD	MET	138	11.111	23.089	17.711	1.00	26.01	APEP
ATOM	1076	CE	MET	138	12.066	21.673	18.272	1.00	23.00	APEP
ATOM	1077	C	MET	138	7.755	23.665	21.768	1.00	15.35	APEP
ATOM	1078	O	MET	138	7.447	22.650	22.395	1.00	15.78	APEP
ATOM	1079	N	TRP	139	8.069	24.824	22.346	1.00	13.56	APEP
ATOM	1080	CA	TRP	139	8.069	25.035	23.791	1.00	10.21	APEP
ATOM	1081	CB	TRP	139	8.700	26.395	24.122	1.00	6.88	APEP
ATOM	1082	CG	TRP	139	10.112	26.582	23.589	1.00	7.62	APEP
ATOM	1083	CD2	TRP	139	10.746	27.821	23.220	1.00	4.45	APEP
ATOM	1084	CE2	TRP	139	12.051	27.507	22.784	1.00	4.04	APEP
ATOM	1085	CE3	TRP	139	10.335	29.160	23.214	1.00	4.82	APEP
ATOM	1086	CD1	TRP	139	11.037	25.606	23.367	1.00	6.61	APEP
ATOM	1087	NE1	TRP	139	12.203	26.151	22.886	1.00	5.06	APEP
ATOM	1088	CZ2	TRP	139	12.955	28.490	22.347	1.00	2.98	APEP
ATOM	1089	CZ3	TRP	139	11.229	30.137	22.778	1.00	2.00	APEP
ATOM	1090	CH2	TRP	139	12.525	29.795	22.351	1.00	4.18	APEP
ATOM	1091	C	TRP	139	6.628	24.997	24.312	1.00	11.53	APEP
ATOM	1092	O	TRP	139	6.350	24.419	25.365	1.00	11.91	APEP
ATOM	1093	N	GLU	140	5.723	25.622	23.557	1.00	11.23	APEP
ATOM	1094	CA	GLU	140	4.306	25.690	23.890	1.00	10.95	APEP
ATOM	1095	CB	GLU	140	3.538	26.427	22.798	1.00	9.60	APEP
ATOM	1096	CG	GLU	140	3.622	27.919	22.834	1.00	7.49	APEP
ATOM	1097	CD	GLU	140	2.893	28.544	21.666	1.00	8.80	APEP
ATOM	1098	OE1	GLU	140	1.937	27.921	21.150	1.00	11.85	APEP
ATOM	1099	OE2	GLU	140	3.277	29.654	21.259	1.00	12.42	APEP
ATOM	1100	C	GLU	140	3.672	24.321	24.038	1.00	11.65	APEP
ATOM	1101	O	GLU	140	2.891	24.089	24.960	1.00	13.93	APEP
ATOM	1102	N	ASP	141	3.993	23.423	23.112	1.00	12.05	APEP
ATOM	1103	CA	ASP	141	3.433	22.078	23.106	1.00	13.22	APEP
ATOM	1104	CB	ASP	141	3.850	21.346	21.833	1.00	12.72	APEP

ATOM	1105	CG	ASP	141	3.200	21.923	20.601	1.00	13.07	APEP
ATOM	1106	OD1	ASP	141	2.240	22.706	20.747	1.00	12.10	APEP
ATOM	1107	OD2	ASP	141	3.646	21.599	19.484	1.00	16.74	APEP
ATOM	1108	C	ASP	141	3.782	21.235	24.320	1.00	13.83	APEP
ATOM	1109	O	ASP	141	3.199	20.172	24.530	1.00	13.83	APEP
ATOM	1110	N	GLU	142	4.726	21.705	25.124	1.00	14.37	APEP
ATOM	1111	CA	GLU	142	5.110	20.974	26.323	1.00	13.86	APEP
ATOM	1112	CB	GLU	142	6.335	21.626	26.974	1.00	13.22	APEP
ATOM	1113	CG	GLU	142	7.619	21.449	26.158	1.00	13.31	APEP
ATOM	1114	CD	GLU	142	8.866	21.896	26.889	1.00	11.68	APEP
ATOM	1115	OE1	GLU	142	8.749	22.706	27.829	1.00	14.70	APEP
ATOM	1116	OE2	GLU	142	9.968	21.439	26.523	1.00	10.37	APEP
ATOM	1117	C	GLU	142	3.937	20.957	27.301	1.00	14.65	APEP
ATOM	1118	O	GLU	142	3.819	20.049	28.120	1.00	16.37	APEP
ATOM	1119	N	VAL	143	3.063	21.954	27.197	1.00	14.52	APEP
ATOM	1120	CA	VAL	143	1.904	22.071	28.084	1.00	14.56	APEP
ATOM	1121	CB	VAL	143	0.970	23.221	27.644	1.00	14.44	APEP
ATOM	1122	CG1	VAL	143	0.210	22.834	26.376	1.00	12.33	APEP
ATOM	1123	CG2	VAL	143	-0.005	23.548	28.769	1.00	10.75	APEP
ATOM	1124	C	VAL	143	1.057	20.809	28.237	1.00	16.78	APEP
ATOM	1125	O	VAL	143	0.399	20.631	29.258	1.00	16.28	APEP
ATOM	1126	N	LYS	144	1.059	19.942	27.228	1.00	17.87	APEP
ATOM	1127	CA	LYS	144	0.281	18.706	27.293	1.00	19.21	APEP
ATOM	1128	CB	LYS	144	0.257	18.025	25.913	1.00	21.23	APEP
ATOM	1129	CG	LYS	144	1.602	17.471	25.452	1.00	23.20	APEP
ATOM	1130	CD	LYS	144	1.578	15.949	25.346	1.00	25.43	APEP
ATOM	1131	CE	LYS	144	2.739	15.423	24.506	1.00	25.49	APEP
ATOM	1132	NZ	LYS	144	2.960	16.244	23.282	1.00	24.96	APEP
ATOM	1133	C	LYS	144	0.852	17.746	28.350	1.00	18.76	APEP
ATOM	1134	O	LYS	144	0.188	16.794	28.776	1.00	18.94	APEP
ATOM	1135	N	ASP	145	2.080	18.008	28.774	1.00	17.39	APEP
ATOM	1136	CA	ASP	145	2.743	17.180	29.778	1.00	18.35	APEP
ATOM	1137	CB	ASP	145	4.199	16.916	29.364	1.00	20.30	APEP
ATOM	1138	CG	ASP	145	4.316	15.942	28.195	1.00	20.35	APEP
ATOM	1139	OD1	ASP	145	3.374	15.153	27.959	1.00	22.15	APEP
ATOM	1140	OD2	ASP	145	5.359	15.966	27.510	1.00	21.64	APEP
ATOM	1141	C	ASP	145	2.714	17.829	31.173	1.00	17.37	APEP
ATOM	1142	O	ASP	145	3.069	17.192	32.164	1.00	15.03	APEP
ATOM	1143	N	TYR	146	2.284	19.090	31.240	1.00	17.14	APEP
ATOM	1144	CA	TYR	146	2.200	19.821	32.506	1.00	15.39	APEP
ATOM	1145	CB	TYR	146	2.368	21.320	32.264	1.00	14.76	APEP
ATOM	1146	CG	TYR	146	2.696	22.071	33.533	1.00	15.28	APEP
ATOM	1147	CD1	TYR	146	3.992	22.053	34.065	1.00	14.62	APEP
ATOM	1148	CE1	TYR	146	4.286	22.682	35.277	1.00	13.76	APEP
ATOM	1149	CD2	TYR	146	1.705	22.743	34.242	1.00	14.99	APEP
ATOM	1150	CE2	TYR	146	1.991	23.377	35.457	1.00	13.01	APEP
ATOM	1151	CZ	TYR	146	3.281	23.340	35.964	1.00	12.87	APEP
ATOM	1152	OH	TYR	146	3.563	23.958	37.162	1.00	12.82	APEP
ATOM	1153	C	TYR	146	0.906	19.580	33.294	1.00	16.31	APEP
ATOM	1154	O	TYR	146	-0.202	19.837	32.804	1.00	15.92	APEP
ATOM	1155	N	ASN	147	1.062	19.097	34.525	1.00	15.90	APEP
ATOM	1156	CA	ASN	147	-0.066	18.792	35.415	1.00	17.53	APEP
ATOM	1157	CB	ASN	147	0.265	17.551	36.248	1.00	17.07	APEP
ATOM	1158	CG	ASN	147	-0.851	17.172	37.193	1.00	18.14	APEP
ATOM	1159	OD1	ASN	147	-1.885	17.835	37.242	1.00	19.58	APEP
ATOM	1160	ND2	ASN	147	-0.651	16.096	37.949	1.00	15.35	APEP
ATOM	1161	C	ASN	147	-0.405	19.957	36.355	1.00	17.15	APEP

ATOM	1162	O	ASN	147	0.289	20.196	37.334	1.00	16.44	APEP
ATOM	1163	N	PRO	148	-1.499	20.677	36.082	1.00	17.84	APEP
ATOM	1164	CD	PRO	148	-2.462	20.508	34.981	1.00	16.74	APEP
ATOM	1165	CA	PRO	148	-1.854	21.805	36.951	1.00	19.69	APEP
ATOM	1166	CB	PRO	148	-2.982	22.504	36.189	1.00	17.29	APEP
ATOM	1167	CG	PRO	148	-3.588	21.436	35.367	1.00	17.85	APEP
ATOM	1168	C	PRO	148	-2.246	21.467	38.395	1.00	21.26	APEP
ATOM	1169	O	PRO	148	-2.040	22.285	39.289	1.00	22.85	APEP
ATOM	1170	N	LYS	149	-2.802	20.275	38.624	1.00	23.35	APEP
ATOM	1171	CA	LYS	149	-3.219	19.862	39.970	1.00	25.52	APEP
ATOM	1172	CB	LYS	149	-4.006	18.546	39.917	1.00	25.99	APEP
ATOM	1173	CG	LYS	149	-5.036	18.477	38.800	1.00	29.64	APEP
ATOM	1174	CD	LYS	149	-6.438	18.800	39.307	1.00	30.49	APEP
ATOM	1175	CE	LYS	149	-7.162	19.775	38.382	1.00	30.51	APEP
ATOM	1176	NZ	LYS	149	-8.401	20.351	39.004	1.00	30.17	APEP
ATOM	1177	C	LYS	149	-2.041	19.704	40.929	1.00	26.61	APEP
ATOM	1178	O	LYS	149	-2.153	19.082	41.993	1.00	27.31	APEP
ATOM	1179	N	LYS	150	-0.905	20.271	40.559	1.00	26.69	APEP
ATOM	1180	CA	LYS	150	0.262	20.186	41.408	1.00	27.83	APEP
ATOM	1181	CB	LYS	150	0.904	18.795	41.260	1.00	26.22	APEP
ATOM	1182	CG	LYS	150	2.205	18.718	40.495	1.00	25.69	APEP
ATOM	1183	CD	LYS	150	2.416	17.320	39.908	1.00	25.52	APEP
ATOM	1184	CE	LYS	150	2.140	16.214	40.922	1.00	24.65	APEP
ATOM	1185	NZ	LYS	150	0.695	15.847	40.971	1.00	23.30	APEP
ATOM	1186	C	LYS	150	1.218	21.320	41.062	1.00	29.22	APEP
ATOM	1187	O	LYS	150	1.504	21.577	39.895	1.00	31.30	APEP
ATOM	1188	N	LYS	151	1.681	22.023	42.088	1.00	30.63	APEP
ATOM	1189	CA	LYS	151	2.581	23.148	41.896	1.00	29.98	APEP
ATOM	1190	CB	LYS	151	3.134	23.622	43.244	1.00	30.75	APEP
ATOM	1191	CG	LYS	151	2.308	24.738	43.888	1.00	32.85	APEP
ATOM	1192	CD	LYS	151	2.605	26.093	43.246	1.00	32.11	APEP
ATOM	1193	CE	LYS	151	1.512	27.104	43.562	1.00	30.61	APEP
ATOM	1194	NZ	LYS	151	2.061	28.331	44.196	1.00	27.07	APEP
ATOM	1195	C	LYS	151	3.720	22.801	40.956	1.00	28.42	APEP
ATOM	1196	O	LYS	151	3.984	21.633	40.685	1.00	28.09	APEP
ATOM	1197	N	PHE	152	4.377	23.842	40.460	1.00	28.09	APEP
ATOM	1198	CA	PHE	152	5.494	23.719	39.539	1.00	27.23	APEP
ATOM	1199	CB	PHE	152	6.138	25.098	39.349	1.00	23.04	APEP
ATOM	1200	CG	PHE	152	7.486	25.064	38.687	1.00	21.73	APEP
ATOM	1201	CD1	PHE	152	7.595	24.951	37.307	1.00	19.77	APEP
ATOM	1202	CD2	PHE	152	8.646	25.171	39.442	1.00	21.08	APEP
ATOM	1203	CE1	PHE	152	8.833	24.948	36.688	1.00	18.18	APEP
ATOM	1204	CE2	PHE	152	9.894	25.169	38.832	1.00	20.33	APEP
ATOM	1205	CZ	PHE	152	9.986	25.057	37.447	1.00	20.45	APEP
ATOM	1206	C	PHE	152	6.543	22.708	39.996	1.00	28.98	APEP
ATOM	1207	O	PHE	152	6.821	21.736	39.293	1.00	29.79	APEP
ATOM	1208	N	SER	153	7.112	22.940	41.176	1.00	30.67	APEP
ATOM	1209	CA	SER	153	8.162	22.084	41.735	1.00	32.34	APEP
ATOM	1210	CB	SER	153	8.313	22.357	43.234	1.00	33.66	APEP
ATOM	1211	OG	SER	153	9.539	21.834	43.713	1.00	35.63	APEP
ATOM	1212	C	SER	153	7.990	20.581	41.522	1.00	31.88	APEP
ATOM	1213	O	SER	153	8.977	19.859	41.342	1.00	29.52	APEP
ATOM	1214	N	GLY	154	6.744	20.113	41.547	1.00	32.09	APEP
ATOM	1215	CA	GLY	154	6.488	18.694	41.373	1.00	31.42	APEP
ATOM	1216	C	GLY	154	6.124	18.282	39.962	1.00	31.52	APEP
ATOM	1217	O	GLY	154	5.317	17.371	39.771	1.00	31.82	APEP
ATOM	1218	N	ASN	155	6.719	18.941	38.973	1.00	30.65	APEP

ATOM	1219	CA	ASN	155	6.448	18.635	37.573	1.00	28.76	APEP
ATOM	1220	CB	ASN	155	5.796	19.842	36.893	1.00	27.64	APEP
ATOM	1221	CG	ASN	155	4.332	19.614	36.579	1.00	26.54	APEP
ATOM	1222	OD1	ASN	155	3.991	18.873	35.652	1.00	25.37	APEP
ATOM	1223	ND2	ASN	155	3.455	20.248	37.354	1.00	23.70	APEP
ATOM	1224	C	ASN	155	7.729	18.257	36.833	1.00	29.00	APEP
ATOM	1225	O	ASN	155	8.828	18.636	37.242	1.00	28.36	APEP
ATOM	1226	N	ASP	156	7.580	17.507	35.744	1.00	28.55	APEP
ATOM	1227	CA	ASP	156	8.714	17.071	34.933	1.00	28.23	APEP
ATOM	1228	CB	ASP	156	8.219	16.108	33.848	1.00	29.34	APEP
ATOM	1229	CG	ASP	156	9.251	15.058	33.474	1.00	30.85	APEP
ATOM	1230	OD1	ASP	156	8.915	13.855	33.515	1.00	30.64	APEP
ATOM	1231	OD2	ASP	156	10.396	15.434	33.133	1.00	32.06	APEP
ATOM	1232	C	ASP	156	9.399	18.281	34.284	1.00	27.76	APEP
ATOM	1233	O	ASP	156	8.971	18.746	33.230	1.00	27.49	APEP
ATOM	1234	N	PHE	157	10.464	18.789	34.897	1.00	28.19	APEP
ATOM	1235	CA	PHE	157	11.129	19.951	34.326	1.00	29.10	APEP
ATOM	1236	CB	PHE	157	11.963	20.705	35.387	1.00	32.07	APEP
ATOM	1237	CG	PHE	157	13.062	19.893	36.038	1.00	35.87	APEP
ATOM	1238	CD1	PHE	157	14.226	19.555	35.330	1.00	36.99	APEP
ATOM	1239	CD2	PHE	157	12.982	19.555	37.397	1.00	35.92	APEP
ATOM	1240	CE1	PHE	157	15.297	18.901	35.966	1.00	36.12	APEP
ATOM	1241	CE2	PHE	157	14.047	18.902	38.044	1.00	36.27	APEP
ATOM	1242	CZ	PHE	157	15.208	18.577	37.323	1.00	36.25	APEP
ATOM	1243	C	PHE	157	11.967	19.639	33.100	1.00	28.86	APEP
ATOM	1244	O	PHE	157	12.423	20.543	32.400	1.00	28.11	APEP
ATOM	1245	N	LEU	158	12.158	18.357	32.824	1.00	28.54	APEP
ATOM	1246	CA	LEU	158	12.928	17.962	31.653	1.00	28.65	APEP
ATOM	1247	CB	LEU	158	13.685	16.657	31.922	1.00	29.80	APEP
ATOM	1248	CG	LEU	158	15.217	16.660	31.842	1.00	28.78	APEP
ATOM	1249	CD1	LEU	158	15.688	15.223	31.692	1.00	29.48	APEP
ATOM	1250	CD2	LEU	158	15.706	17.507	30.669	1.00	26.31	APEP
ATOM	1251	C	LEU	158	11.962	17.773	30.488	1.00	27.95	APEP
ATOM	1252	O	LEU	158	12.375	17.501	29.366	1.00	30.04	APEP
ATOM	1253	N	LYS	159	10.671	17.932	30.763	1.00	26.29	APEP
ATOM	1254	CA	LYS	159	9.654	17.774	29.734	1.00	24.09	APEP
ATOM	1255	CB	LYS	159	8.801	16.542	30.039	1.00	23.00	APEP
ATOM	1256	CG	LYS	159	9.619	15.265	30.203	1.00	24.44	APEP
ATOM	1257	CD	LYS	159	8.749	14.035	30.403	1.00	23.01	APEP
ATOM	1258	CE	LYS	159	7.414	14.154	29.691	1.00	22.17	APEP
ATOM	1259	NZ	LYS	159	6.363	13.362	30.384	1.00	21.65	APEP
ATOM	1260	C	LYS	159	8.756	19.000	29.595	1.00	22.82	APEP
ATOM	1261	O	LYS	159	8.099	19.182	28.566	1.00	20.81	APEP
ATOM	1262	N	THR	160	8.731	19.845	30.623	1.00	21.93	APEP
ATOM	1263	CA	THR	160	7.889	21.041	30.590	1.00	19.90	APEP
ATOM	1264	CB	THR	160	6.684	20.884	31.554	1.00	17.71	APEP
ATOM	1265	OG1	THR	160	7.163	20.721	32.894	1.00	17.45	APEP
ATOM	1266	CG2	THR	160	5.856	19.670	31.182	1.00	14.19	APEP
ATOM	1267	C	THR	160	8.619	22.352	30.921	1.00	19.16	APEP
ATOM	1268	O	THR	160	8.005	23.419	30.937	1.00	20.80	APEP
ATOM	1269	N	GLY	161	9.925	22.270	31.160	1.00	17.07	APEP
ATOM	1270	CA	GLY	161	10.707	23.446	31.506	1.00	15.84	APEP
ATOM	1271	C	GLY	161	10.629	24.679	30.616	1.00	15.32	APEP
ATOM	1272	O	GLY	161	10.825	25.796	31.093	1.00	15.24	APEP
ATOM	1273	N	HIS	162	10.356	24.498	29.329	1.00	15.82	APEP
ATOM	1274	CA	HIS	162	10.274	25.637	28.421	1.00	14.73	APEP
ATOM	1275	CB	HIS	162	10.587	25.193	26.995	1.00	17.02	APEP

ATOM	1276	CG	HIS	162	11.979	24.675	26.823	1.00	20.85	APEP
ATOM	1277	CD2	HIS	162	13.162	25.120	27.308	1.00	21.78	APEP
ATOM	1278	ND1	HIS	162	12.268	23.554	26.076	1.00	23.37	APEP
ATOM	1279	CE1	HIS	162	13.572	23.333	26.107	1.00	24.65	APEP
ATOM	1280	NE2	HIS	162	14.136	24.269	26.848	1.00	24.25	APEP
ATOM	1281	C	HIS	162	8.893	26.277	28.486	1.00	13.27	APEP
ATOM	1282	O	HIS	162	8.753	27.497	28.413	1.00	11.88	APEP
ATOM	1283	N	TYR	163	7.875	25.442	28.628	1.00	11.72	APEP
ATOM	1284	CA	TYR	163	6.509	25.926	28.733	1.00	11.23	APEP
ATOM	1285	CB	TYR	163	5.550	24.750	28.924	1.00	10.40	APEP
ATOM	1286	CG	TYR	163	4.271	25.122	29.653	1.00	10.24	APEP
ATOM	1287	CD1	TYR	163	3.369	26.028	29.095	1.00	10.55	APEP
ATOM	1288	CE1	TYR	163	2.196	26.378	29.759	1.00	9.30	APEP
ATOM	1289	CD2	TYR	163	3.970	24.576	30.898	1.00	6.58	APEP
ATOM	1290	CE2	TYR	163	2.802	24.920	31.571	1.00	8.87	APEP
ATOM	1291	CZ	TYR	163	1.918	25.818	30.995	1.00	10.63	APEP
ATOM	1292	OH	TYR	163	0.745	26.143	31.635	1.00	10.74	APEP
ATOM	1293	C	TYR	163	6.387	26.863	29.937	1.00	10.06	APEP
ATOM	1294	O	TYR	163	5.919	27.994	29.820	1.00	10.02	APEP
ATOM	1295	N	THR	164	6.830	26.382	31.093	1.00	9.35	APEP
ATOM	1296	CA	THR	164	6.740	27.143	32.335	1.00	8.02	APEP
ATOM	1297	CB	THR	164	7.259	26.301	33.511	1.00	6.41	APEP
ATOM	1298	OG1	THR	164	8.571	25.811	33.209	1.00	5.81	APEP
ATOM	1299	CG2	THR	164	6.312	25.111	33.750	1.00	2.00	APEP
ATOM	1300	C	THR	164	7.422	28.513	32.327	1.00	8.62	APEP
ATOM	1301	O	THR	164	6.962	29.433	33.010	1.00	8.07	APEP
ATOM	1302	N	GLN	165	8.508	28.663	31.570	1.00	8.53	APEP
ATOM	1303	CA	GLN	165	9.183	29.964	31.499	1.00	7.81	APEP
ATOM	1304	CB	GLN	165	10.598	29.837	30.930	1.00	7.00	APEP
ATOM	1305	CG	GLN	165	11.241	31.179	30.604	1.00	7.27	APEP
ATOM	1306	CD	GLN	165	11.537	32.023	31.840	1.00	7.94	APEP
ATOM	1307	OE1	GLN	165	12.631	31.963	32.407	1.00	7.98	APEP
ATOM	1308	NE2	GLN	165	10.566	32.815	32.257	1.00	5.43	APEP
ATOM	1309	C	GLN	165	8.370	30.902	30.609	1.00	7.95	APEP
ATOM	1310	O	GLN	165	8.363	32.113	30.811	1.00	8.38	APEP
ATOM	1311	N	MET	166	7.683	30.330	29.625	1.00	7.26	APEP
ATOM	1312	CA	MET	166	6.859	31.118	28.715	1.00	8.35	APEP
ATOM	1313	CB	MET	166	6.377	30.253	27.553	1.00	7.91	APEP
ATOM	1314	CG	MET	166	7.245	30.356	26.309	1.00	7.80	APEP
ATOM	1315	SD	MET	166	6.486	29.500	24.931	1.00	12.38	APEP
ATOM	1316	CE	MET	166	5.508	30.823	24.207	1.00	11.75	APEP
ATOM	1317	C	MET	166	5.654	31.746	29.409	1.00	6.47	APEP
ATOM	1318	O	MET	166	5.313	32.890	29.128	1.00	7.34	APEP
ATOM	1319	N	VAL	167	5.011	31.009	30.314	1.00	7.21	APEP
ATOM	1320	CA	VAL	167	3.847	31.550	31.018	1.00	7.06	APEP
ATOM	1321	CB	VAL	167	2.676	30.526	31.065	1.00	7.32	APEP
ATOM	1322	CG1	VAL	167	2.295	30.107	29.654	1.00	3.76	APEP
ATOM	1323	CG2	VAL	167	3.048	29.321	31.901	1.00	5.77	APEP
ATOM	1324	C	VAL	167	4.125	32.046	32.444	1.00	8.39	APEP
ATOM	1325	O	VAL	167	3.200	32.211	33.231	1.00	8.06	APEP
ATOM	1326	N	TRP	168	5.396	32.290	32.767	1.00	9.11	APEP
ATOM	1327	CA	TRP	168	5.793	32.784	34.089	1.00	8.50	APEP
ATOM	1328	CB	TRP	168	7.320	32.745	34.232	1.00	7.79	APEP
ATOM	1329	CG	TRP	168	7.806	32.690	35.657	1.00	9.67	APEP
ATOM	1330	CD2	TRP	168	7.960	31.519	36.474	1.00	8.72	APEP
ATOM	1331	CE2	TRP	168	8.452	31.946	37.725	1.00	10.18	APEP
ATOM	1332	CE3	TRP	168	7.730	30.153	36.268	1.00	10.13	APEP

ATOM	1333	CD1	TRP	168	8.200	33.747	36.430	1.00	8.39	APEP
ATOM	1334	NE1	TRP	168	8.589	33.309	37.670	1.00	8.61	APEP
ATOM	1335	CZ2	TRP	168	8.722	31.054	38.769	1.00	8.53	APEP
ATOM	1336	CZ3	TRP	168	7.998	29.265	37.305	1.00	6.40	APEP
ATOM	1337	CH2	TRP	168	8.488	29.721	38.539	1.00	9.27	APEP
ATOM	1338	C	TRP	168	5.294	34.213	34.275	1.00	8.27	APEP
ATOM	1339	O	TRP	168	5.782	35.136	33.627	1.00	8.24	APEP
ATOM	1340	N	ALA	169	4.324	34.392	35.167	1.00	7.85	APEP
ATOM	1341	CA	ALA	169	3.734	35.704	35.415	1.00	7.42	APEP
ATOM	1342	CB	ALA	169	2.668	35.585	36.476	1.00	6.86	APEP
ATOM	1343	C	ALA	169	4.715	36.805	35.797	1.00	9.10	APEP
ATOM	1344	O	ALA	169	4.525	37.968	35.433	1.00	10.62	APEP
ATOM	1345	N	ASN	170	5.758	36.436	36.531	1.00	9.55	APEP
ATOM	1346	CA	ASN	170	6.760	37.392	36.990	1.00	10.81	APEP
ATOM	1347	CB	ASN	170	7.557	36.792	38.158	1.00	10.24	APEP
ATOM	1348	CG	ASN	170	6.907	37.057	39.513	1.00	11.09	APEP
ATOM	1349	OD1	ASN	170	5.758	37.497	39.598	1.00	11.64	APEP
ATOM	1350	ND2	ASN	170	7.643	36.783	40.578	1.00	14.04	APEP
ATOM	1351	C	ASN	170	7.716	37.859	35.887	1.00	10.96	APEP
ATOM	1352	O	ASN	170	8.317	38.918	35.999	1.00	10.88	APEP
ATOM	1353	N	THR	171	7.866	37.071	34.830	1.00	10.69	APEP
ATOM	1354	CA	THR	171	8.741	37.472	33.733	1.00	9.90	APEP
ATOM	1355	CB	THR	171	9.002	36.308	32.757	1.00	8.82	APEP
ATOM	1356	OG1	THR	171	9.738	35.278	33.430	1.00	10.00	APEP
ATOM	1357	CG2	THR	171	9.793	36.790	31.541	1.00	6.32	APEP
ATOM	1358	C	THR	171	8.026	38.592	32.992	1.00	10.43	APEP
ATOM	1359	O	THR	171	6.842	38.468	32.669	1.00	11.58	APEP
ATOM	1360	N	LYS	172	8.736	39.680	32.715	1.00	11.90	APEP
ATOM	1361	CA	LYS	172	8.131	40.818	32.027	1.00	12.07	APEP
ATOM	1362	CB	LYS	172	8.176	42.054	32.934	1.00	13.23	APEP
ATOM	1363	CG	LYS	172	7.424	41.893	34.261	1.00	16.65	APEP
ATOM	1364	CD	LYS	172	5.905	41.830	34.074	1.00	18.48	APEP
ATOM	1365	CE	LYS	172	5.354	43.044	33.312	1.00	21.38	APEP
ATOM	1366	NZ	LYS	172	4.386	43.852	34.117	1.00	19.33	APEP
ATOM	1367	C	LYS	172	8.751	41.166	30.677	1.00	9.74	APEP
ATOM	1368	O	LYS	172	8.122	41.834	29.856	1.00	9.87	APEP
ATOM	1369	N	GLU	173	9.982	40.719	30.450	1.00	11.94	APEP
ATOM	1370	CA	GLU	173	10.684	41.005	29.198	1.00	13.45	APEP
ATOM	1371	CB	GLU	173	11.812	42.014	29.438	1.00	16.52	APEP
ATOM	1372	CG	GLU	173	11.752	42.721	30.778	1.00	21.67	APEP
ATOM	1373	CD	GLU	173	11.695	44.220	30.618	1.00	23.96	APEP
ATOM	1374	OE1	GLU	173	11.727	44.679	29.455	1.00	24.88	APEP
ATOM	1375	OE2	GLU	173	11.621	44.935	31.643	1.00	28.83	APEP
ATOM	1376	C	GLU	173	11.280	39.765	28.531	1.00	10.86	APEP
ATOM	1377	O	GLU	173	11.622	38.790	29.199	1.00	10.81	APEP
ATOM	1378	N	VAL	174	11.404	39.830	27.209	1.00	10.16	APEP
ATOM	1379	CA	VAL	174	11.968	38.741	26.416	1.00	9.96	APEP
ATOM	1380	CB	VAL	174	10.856	37.811	25.846	1.00	9.93	APEP
ATOM	1381	CG1	VAL	174	10.099	38.519	24.740	1.00	7.96	APEP
ATOM	1382	CG2	VAL	174	11.460	36.508	25.323	1.00	7.52	APEP
ATOM	1383	C	VAL	174	12.790	39.316	25.258	1.00	11.17	APEP
ATOM	1384	O	VAL	174	12.485	40.383	24.728	1.00	11.23	APEP
ATOM	1385	N	GLY	175	13.845	38.605	24.886	1.00	10.40	APEP
ATOM	1386	CA	GLY	175	14.692	39.045	23.797	1.00	8.75	APEP
ATOM	1387	C	GLY	175	15.337	37.813	23.211	1.00	9.59	APEP
ATOM	1388	O	GLY	175	15.882	36.991	23.949	1.00	6.65	APEP
ATOM	1389	N	CYS	176	15.291	37.685	21.885	1.00	8.02	APEP

ATOM	1390	CA	CYS	176	15.853	36.511	21.226	1.00	7.90	APEP
ATOM	1391	C	CYS	176	16.940	36.771	20.186	1.00	7.85	APEP
ATOM	1392	O	CYS	176	17.114	37.893	19.693	1.00	6.33	APEP
ATOM	1393	CB	CYS	176	14.721	35.701	20.582	1.00	6.21	APEP
ATOM	1394	SG	CYS	176	13.249	35.553	21.641	1.00	9.41	APEP
ATOM	1395	N	GLY	177	17.672	35.703	19.880	1.00	8.22	APEP
ATOM	1396	CA	GLY	177	18.737	35.744	18.895	1.00	10.14	APEP
ATOM	1397	C	GLY	177	18.618	34.466	18.085	1.00	10.70	APEP
ATOM	1398	O	GLY	177	18.182	33.446	18.623	1.00	8.40	APEP
ATOM	1399	N	SER	178	18.983	34.509	16.806	1.00	9.75	APEP
ATOM	1400	CA	SER	178	18.881	33.325	15.959	1.00	9.62	APEP
ATOM	1401	CB	SER	178	17.523	33.305	15.247	1.00	12.13	APEP
ATOM	1402	OG	SER	178	17.614	33.902	13.964	1.00	15.65	APEP
ATOM	1403	C	SER	178	19.999	33.227	14.921	1.00	8.56	APEP
ATOM	1404	O	SER	178	20.597	34.231	14.532	1.00	5.09	APEP
ATOM	1405	N	ILE	179	20.270	32.001	14.482	1.00	9.37	APEP
ATOM	1406	CA	ILE	179	21.310	31.744	13.498	1.00	9.01	APEP
ATOM	1407	CB	ILE	179	22.673	31.531	14.181	1.00	8.43	APEP
ATOM	1408	CG2	ILE	179	22.625	30.296	15.054	1.00	6.63	APEP
ATOM	1409	CG1	ILE	179	23.774	31.415	13.122	1.00	7.53	APEP
ATOM	1410	CD1	ILE	179	25.093	32.035	13.535	1.00	8.62	APEP
ATOM	1411	C	ILE	179	20.980	30.517	12.650	1.00	10.87	APEP
ATOM	1412	O	ILE	179	20.506	29.497	13.158	1.00	10.47	APEP
ATOM	1413	N	LYS	180	21.216	30.632	11.347	1.00	10.60	APEP
ATOM	1414	CA	LYS	180	20.952	29.536	10.430	1.00	11.05	APEP
ATOM	1415	CB	LYS	180	20.077	30.018	9.269	1.00	11.05	APEP
ATOM	1416	CG	LYS	180	18.745	30.596	9.724	1.00	13.07	APEP
ATOM	1417	CD	LYS	180	17.902	31.048	8.543	1.00	15.79	APEP
ATOM	1418	CE	LYS	180	16.580	31.655	8.996	1.00	14.81	APEP
ATOM	1419	NZ	LYS	180	15.620	31.802	7.862	1.00	16.66	APEP
ATOM	1420	C	LYS	180	22.291	29.029	9.920	1.00	10.53	APEP
ATOM	1421	O	LYS	180	23.137	29.817	9.505	1.00	12.20	APEP
ATOM	1422	N	TYR	181	22.490	27.716	9.955	1.00	9.11	APEP
ATOM	1423	CA	TYR	181	23.756	27.166	9.510	1.00	8.14	APEP
ATOM	1424	CB	TYR	181	24.786	27.300	10.633	1.00	6.13	APEP
ATOM	1425	CG	TYR	181	24.460	26.483	11.863	1.00	8.18	APEP
ATOM	1426	CD1	TYR	181	24.984	25.201	12.027	1.00	9.63	APEP
ATOM	1427	CE1	TYR	181	24.706	24.450	13.172	1.00	8.66	APEP
ATOM	1428	CD2	TYR	181	23.643	26.999	12.876	1.00	8.34	APEP
ATOM	1429	CE2	TYR	181	23.360	26.257	14.020	1.00	7.46	APEP
ATOM	1430	CZ	TYR	181	23.896	24.986	14.161	1.00	8.38	APEP
ATOM	1431	OH	TYR	181	23.634	24.244	15.293	1.00	8.05	APEP
ATOM	1432	C	TYR	181	23.695	25.719	9.022	1.00	7.08	APEP
ATOM	1433	O	TYR	181	22.728	25.001	9.262	1.00	7.97	APEP
ATOM	1434	N	ILE	182	24.743	25.303	8.323	1.00	8.25	APEP
ATOM	1435	CA	ILE	182	24.814	23.948	7.804	1.00	7.94	APEP
ATOM	1436	CB	ILE	182	25.011	23.959	6.293	1.00	9.43	APEP
ATOM	1437	CG2	ILE	182	24.641	22.599	5.713	1.00	9.49	APEP
ATOM	1438	CG1	ILE	182	24.147	25.065	5.680	1.00	9.61	APEP
ATOM	1439	CD1	ILE	182	24.502	25.429	4.278	1.00	7.05	APEP
ATOM	1440	C	ILE	182	25.961	23.184	8.445	1.00	8.67	APEP
ATOM	1441	O	ILE	182	27.112	23.588	8.333	1.00	8.62	APEP
ATOM	1442	N	GLN	183	25.642	22.084	9.122	1.00	6.39	APEP
ATOM	1443	CA	GLN	183	26.657	21.271	9.776	1.00	7.60	APEP
ATOM	1444	CB	GLN	183	26.485	21.313	11.304	1.00	8.26	APEP
ATOM	1445	CG	GLN	183	27.184	20.160	12.059	1.00	11.17	APEP
ATOM	1446	CD	GLN	183	26.842	20.105	13.560	1.00	10.23	APEP

ATOM	1447	OE1	GLN	183	25.927	20.779	14.029	1.00	13.96	APEP
ATOM	1448	NE2	GLN	183	27.578	19.293	14.304	1.00	9.47	APEP
ATOM	1449	C	GLN	183	26.603	19.824	9.308	1.00	6.95	APEP
ATOM	1450	O	GLN	183	25.653	19.096	9.602	1.00	4.41	APEP
ATOM	1451	N	GLU	184	27.624	19.404	8.576	1.00	6.92	APEP
ATOM	1452	CA	GLU	184	27.685	18.025	8.123	1.00	7.66	APEP
ATOM	1453	CB	GLU	184	27.885	17.120	9.349	1.00	9.14	APEP
ATOM	1454	CG	GLU	184	29.110	17.551	10.172	1.00	8.94	APEP
ATOM	1455	CD	GLU	184	29.207	16.912	11.558	1.00	12.48	APEP
ATOM	1456	OE1	GLU	184	28.235	16.963	12.340	1.00	13.16	APEP
ATOM	1457	OE2	GLU	184	30.278	16.361	11.869	1.00	15.28	APEP
ATOM	1458	C	GLU	184	26.447	17.645	7.316	1.00	6.36	APEP
ATOM	1459	O	GLU	184	25.858	16.581	7.493	1.00	4.35	APEP
ATOM	1460	N	LYS	185	26.089	18.560	6.417	1.00	8.43	APEP
ATOM	1461	CA	LYS	185	24.956	18.451	5.504	1.00	10.57	APEP
ATOM	1462	CB	LYS	185	25.054	17.165	4.685	1.00	12.55	APEP
ATOM	1463	CG	LYS	185	25.705	17.371	3.331	1.00	16.19	APEP
ATOM	1464	CD	LYS	185	26.930	16.498	3.173	1.00	20.18	APEP
ATOM	1465	CE	LYS	185	26.783	15.553	1.990	1.00	22.27	APEP
ATOM	1466	NZ	LYS	185	25.360	15.182	1.744	1.00	25.09	APEP
ATOM	1467	C	LYS	185	23.571	18.567	6.131	1.00	10.92	APEP
ATOM	1468	O	LYS	185	22.566	18.219	5.509	1.00	10.67	APEP
ATOM	1469	N	TRP	186	23.519	19.062	7.362	1.00	10.21	APEP
ATOM	1470	CA	TRP	186	22.250	19.252	8.039	1.00	9.13	APEP
ATOM	1471	CB	TRP	186	22.297	18.677	9.461	1.00	8.92	APEP
ATOM	1472	CG	TRP	186	22.105	17.173	9.563	1.00	7.23	APEP
ATOM	1473	CD2	TRP	186	20.883	16.433	9.380	1.00	7.44	APEP
ATOM	1474	CE2	TRP	186	21.179	15.070	9.624	1.00	7.10	APEP
ATOM	1475	CE3	TRP	186	19.569	16.787	9.036	1.00	7.24	APEP
ATOM	1476	CD1	TRP	186	23.057	16.253	9.895	1.00	7.55	APEP
ATOM	1477	NE1	TRP	186	22.510	14.991	9.934	1.00	7.21	APEP
ATOM	1478	CZ2	TRP	186	20.209	14.061	9.536	1.00	4.65	APEP
ATOM	1479	CZ3	TRP	186	18.602	15.776	8.950	1.00	5.07	APEP
ATOM	1480	CH2	TRP	186	18.934	14.430	9.198	1.00	4.34	APEP
ATOM	1481	C	TRP	186	22.029	20.757	8.097	1.00	9.22	APEP
ATOM	1482	O	TRP	186	22.895	21.495	8.547	1.00	10.78	APEP
ATOM	1483	N	HIS	187	20.876	21.214	7.622	1.00	11.53	APEP
ATOM	1484	CA	HIS	187	20.555	22.637	7.642	1.00	10.59	APEP
ATOM	1485	CB	HIS	187	19.773	23.005	6.375	1.00	11.28	APEP
ATOM	1486	CG	HIS	187	20.381	22.455	5.119	1.00	12.80	APEP
ATOM	1487	CD2	HIS	187	20.511	21.183	4.674	1.00	13.63	APEP
ATOM	1488	ND1	HIS	187	20.984	23.254	4.170	1.00	14.63	APEP
ATOM	1489	CE1	HIS	187	21.463	22.497	3.198	1.00	14.97	APEP
ATOM	1490	NE2	HIS	187	21.189	21.236	3.480	1.00	14.98	APEP
ATOM	1491	C	HIS	187	19.738	22.910	8.904	1.00	8.99	APEP
ATOM	1492	O	HIS	187	18.636	22.408	9.058	1.00	10.40	APEP
ATOM	1493	N	LYS	188	20.287	23.700	9.817	1.00	10.12	APEP
ATOM	1494	CA	LYS	188	19.593	23.970	11.068	1.00	9.30	APEP
ATOM	1495	CB	LYS	188	20.403	23.413	12.251	1.00	10.66	APEP
ATOM	1496	CG	LYS	188	21.395	22.314	11.909	1.00	7.22	APEP
ATOM	1497	CD	LYS	188	21.627	21.422	13.118	1.00	8.34	APEP
ATOM	1498	CE	LYS	188	22.696	20.369	12.871	1.00	9.03	APEP
ATOM	1499	NZ	LYS	188	23.268	19.865	14.162	1.00	13.31	APEP
ATOM	1500	C	LYS	188	19.289	25.428	11.349	1.00	7.81	APEP
ATOM	1501	O	LYS	188	19.988	26.328	10.890	1.00	9.61	APEP
ATOM	1502	N	HIS	189	18.216	25.646	12.097	1.00	9.34	APEP
ATOM	1503	CA	HIS	189	17.823	26.977	12.532	1.00	9.56	APEP

ATOM	1504	CB	HIS	189	16.391	27.315	12.119	1.00	9.45	APEP
ATOM	1505	CG	HIS	189	16.033	28.756	12.331	1.00	11.25	APEP
ATOM	1506	CD2	HIS	189	16.739	29.777	12.870	1.00	11.06	APEP
ATOM	1507	ND1	HIS	189	14.822	29.291	11.946	1.00	13.10	APEP
ATOM	1508	CE1	HIS	189	14.800	30.579	12.237	1.00	11.47	APEP
ATOM	1509	NE2	HIS	189	15.950	30.900	12.799	1.00	12.33	APEP
ATOM	1510	C	HIS	189	17.911	26.904	14.049	1.00	9.30	APEP
ATOM	1511	O	HIS	189	17.265	26.060	14.671	1.00	9.01	APEP
ATOM	1512	N	TYR	190	18.717	27.781	14.635	1.00	10.00	APEP
ATOM	1513	CA	TYR	190	18.928	27.816	16.080	1.00	9.41	APEP
ATOM	1514	CB	TYR	190	20.433	27.745	16.343	1.00	11.08	APEP
ATOM	1515	CG	TYR	190	20.872	27.618	17.788	1.00	11.54	APEP
ATOM	1516	CD1	TYR	190	20.017	27.124	18.777	1.00	11.05	APEP
ATOM	1517	CE1	TYR	190	20.458	26.983	20.108	1.00	10.64	APEP
ATOM	1518	CD2	TYR	190	22.173	27.971	18.157	1.00	11.80	APEP
ATOM	1519	CE2	TYR	190	22.618	27.835	19.467	1.00	12.63	APEP
ATOM	1520	CZ	TYR	190	21.767	27.342	20.435	1.00	11.58	APEP
ATOM	1521	OH	TYR	190	22.257	27.190	21.713	1.00	12.58	APEP
ATOM	1522	C	TYR	190	18.337	29.076	16.716	1.00	8.19	APEP
ATOM	1523	O	TYR	190	18.803	30.184	16.456	1.00	6.34	APEP
ATOM	1524	N	LEU	191	17.313	28.895	17.549	1.00	7.47	APEP
ATOM	1525	CA	LEU	191	16.656	30.011	18.226	1.00	8.92	APEP
ATOM	1526	CB	LEU	191	15.151	30.001	17.926	1.00	8.75	APEP
ATOM	1527	CG	LEU	191	14.308	31.101	18.599	1.00	11.51	APEP
ATOM	1528	CD1	LEU	191	14.540	32.444	17.916	1.00	9.71	APEP
ATOM	1529	CD2	LEU	191	12.831	30.724	18.541	1.00	9.30	APEP
ATOM	1530	C	LEU	191	16.890	29.996	19.743	1.00	9.50	APEP
ATOM	1531	O	LEU	191	16.667	28.988	20.416	1.00	11.01	APEP
ATOM	1532	N	VAL	192	17.347	31.128	20.266	1.00	9.30	APEP
ATOM	1533	CA	VAL	192	17.629	31.291	21.690	1.00	10.13	APEP
ATOM	1534	CB	VAL	192	19.145	31.536	21.923	1.00	10.96	APEP
ATOM	1535	CG1	VAL	192	19.387	32.044	23.344	1.00	11.81	APEP
ATOM	1536	CG2	VAL	192	19.934	30.267	21.659	1.00	11.43	APEP
ATOM	1537	C	VAL	192	16.875	32.510	22.231	1.00	10.34	APEP
ATOM	1538	O	VAL	192	17.078	33.621	21.745	1.00	10.10	APEP
ATOM	1539	N	CYS	193	16.009	32.315	23.226	1.00	8.18	APEP
ATOM	1540	CA	CYS	193	15.276	33.442	23.810	1.00	8.37	APEP
ATOM	1541	C	CYS	193	15.600	33.609	25.296	1.00	8.44	APEP
ATOM	1542	O	CYS	193	15.514	32.650	26.061	1.00	6.27	APEP
ATOM	1543	CB	CYS	193	13.762	33.258	23.649	1.00	9.08	APEP
ATOM	1544	SG	CYS	193	13.062	33.556	21.985	1.00	8.61	APEP
ATOM	1545	N	ASN	194	15.978	34.826	25.694	1.00	8.32	APEP
ATOM	1546	CA	ASN	194	16.310	35.133	27.086	1.00	7.48	APEP
ATOM	1547	CB	ASN	194	17.582	35.991	27.149	1.00	8.69	APEP
ATOM	1548	CG	ASN	194	18.840	35.190	26.868	1.00	6.67	APEP
ATOM	1549	OD1	ASN	194	18.772	33.995	26.596	1.00	9.58	APEP
ATOM	1550	ND2	ASN	194	19.989	35.843	26.932	1.00	4.71	APEP
ATOM	1551	C	ASN	194	15.140	35.859	27.766	1.00	7.75	APEP
ATOM	1552	O	ASN	194	14.540	36.760	27.175	1.00	5.25	APEP
ATOM	1553	N	TYR	195	14.834	35.475	29.009	1.00	7.83	APEP
ATOM	1554	CA	TYR	195	13.699	36.047	29.749	1.00	7.00	APEP
ATOM	1555	CB	TYR	195	12.736	34.924	30.138	1.00	6.30	APEP
ATOM	1556	CG	TYR	195	12.180	34.180	28.949	1.00	8.42	APEP
ATOM	1557	CD1	TYR	195	12.918	33.175	28.329	1.00	7.89	APEP
ATOM	1558	CE1	TYR	195	12.436	32.510	27.219	1.00	8.54	APEP
ATOM	1559	CD2	TYR	195	10.934	34.501	28.422	1.00	6.50	APEP
ATOM	1560	CE2	TYR	195	10.438	33.835	27.300	1.00	8.99	APEP

ATOM	1561	CZ	TYR	195	11.199	32.837	26.707	1.00	8.08	APEP
ATOM	1562	OH	TYR	195	10.724	32.142	25.617	1.00	8.91	APEP
ATOM	1563	C	TYR	195	14.047	36.860	30.999	1.00	6.48	APEP
ATOM	1564	O	TYR	195	14.840	36.422	31.822	1.00	7.26	APEP
ATOM	1565	N	GLY	196	13.418	38.025	31.154	1.00	6.42	APEP
ATOM	1566	CA	GLY	196	13.709	38.867	32.303	1.00	6.88	APEP
ATOM	1567	C	GLY	196	12.558	39.431	33.131	1.00	8.03	APEP
ATOM	1568	O	GLY	196	11.649	40.075	32.596	1.00	8.29	APEP
ATOM	1569	N	PRO	197	12.541	39.157	34.445	1.00	6.50	APEP
ATOM	1570	CD	PRO	197	11.525	39.698	35.363	1.00	7.41	APEP
ATOM	1571	CA	PRO	197	13.536	38.343	35.153	1.00	6.68	APEP
ATOM	1572	CB	PRO	197	13.300	38.688	36.610	1.00	6.91	APEP
ATOM	1573	CG	PRO	197	11.856	39.041	36.672	1.00	6.39	APEP
ATOM	1574	C	PRO	197	13.266	36.868	34.854	1.00	6.69	APEP
ATOM	1575	O	PRO	197	12.255	36.537	34.238	1.00	6.02	APEP
ATOM	1576	N	SER	198	14.153	35.975	35.286	1.00	7.01	APEP
ATOM	1577	CA	SER	198	13.953	34.557	35.001	1.00	7.41	APEP
ATOM	1578	CB	SER	198	15.233	33.755	35.303	1.00	4.71	APEP
ATOM	1579	OG	SER	198	15.558	33.752	36.682	1.00	11.52	APEP
ATOM	1580	C	SER	198	12.765	33.927	35.717	1.00	7.39	APEP
ATOM	1581	O	SER	198	12.161	34.528	36.605	1.00	6.19	APEP
ATOM	1582	N	GLY	199	12.423	32.716	35.289	1.00	7.86	APEP
ATOM	1583	CA	GLY	199	11.332	31.977	35.893	1.00	8.49	APEP
ATOM	1584	C	GLY	199	11.894	30.622	36.274	1.00	8.12	APEP
ATOM	1585	O	GLY	199	13.110	30.450	36.306	1.00	8.44	APEP
ATOM	1586	N	ASN	200	11.022	29.670	36.570	1.00	9.40	APEP
ATOM	1587	CA	ASN	200	11.433	28.317	36.929	1.00	11.14	APEP
ATOM	1588	CB	ASN	200	12.344	27.742	35.844	1.00	11.38	APEP
ATOM	1589	CG	ASN	200	11.581	27.365	34.591	1.00	12.36	APEP
ATOM	1590	OD1	ASN	200	10.360	27.478	34.550	1.00	13.35	APEP
ATOM	1591	ND2	ASN	200	12.293	26.919	33.566	1.00	10.02	APEP
ATOM	1592	C	ASN	200	12.102	28.177	38.296	1.00	12.89	APEP
ATOM	1593	O	ASN	200	13.019	27.373	38.477	1.00	12.94	APEP
ATOM	1594	N	PHE	201	11.632	28.957	39.262	1.00	14.46	APEP
ATOM	1595	CA	PHE	201	12.157	28.890	40.622	1.00	16.00	APEP
ATOM	1596	CB	PHE	201	11.947	30.224	41.339	1.00	14.75	APEP
ATOM	1597	CG	PHE	201	12.805	31.338	40.811	1.00	13.67	APEP
ATOM	1598	CD1	PHE	201	12.267	32.311	39.982	1.00	13.83	APEP
ATOM	1599	CD2	PHE	201	14.151	31.421	41.157	1.00	17.70	APEP
ATOM	1600	CE1	PHE	201	13.047	33.350	39.505	1.00	14.74	APEP
ATOM	1601	CE2	PHE	201	14.948	32.460	40.685	1.00	17.45	APEP
ATOM	1602	CZ	PHE	201	14.394	33.427	39.857	1.00	17.09	APEP
ATOM	1603	C	PHE	201	11.347	27.786	41.304	1.00	16.58	APEP
ATOM	1604	O	PHE	201	10.124	27.876	41.385	1.00	16.44	APEP
ATOM	1605	N	LYS	202	12.026	26.755	41.797	1.00	18.81	APEP
ATOM	1606	CA	LYS	202	11.351	25.617	42.421	1.00	21.27	APEP
ATOM	1607	CB	LYS	202	12.386	24.588	42.883	1.00	25.00	APEP
ATOM	1608	CG	LYS	202	12.314	23.274	42.101	1.00	29.78	APEP
ATOM	1609	CD	LYS	202	13.215	22.197	42.698	1.00	31.92	APEP
ATOM	1610	CE	LYS	202	12.574	20.816	42.609	1.00	32.51	APEP
ATOM	1611	NZ	LYS	202	12.138	20.304	43.944	1.00	30.68	APEP
ATOM	1612	C	LYS	202	10.365	25.899	43.555	1.00	21.29	APEP
ATOM	1613	O	LYS	202	9.347	25.218	43.676	1.00	22.50	APEP
ATOM	1614	N	ASN	203	10.642	26.894	44.385	1.00	20.90	APEP
ATOM	1615	CA	ASN	203	9.726	27.190	45.485	1.00	22.00	APEP
ATOM	1616	CB	ASN	203	10.520	27.657	46.711	1.00	23.02	APEP
ATOM	1617	CG	ASN	203	11.274	28.953	46.466	1.00	23.60	APEP

ATOM	1618	OD1	ASN	203	11.559	29.698	47.401	1.00	24.95	APEP
ATOM	1619	ND2	ASN	203	11.605	29.223	45.209	1.00	25.38	APEP
ATOM	1620	C	ASN	203	8.656	28.232	45.134	1.00	20.82	APEP
ATOM	1621	O	ASN	203	8.096	28.877	46.025	1.00	20.77	APEP
ATOM	1622	N	GLU	204	8.363	28.384	43.845	1.00	16.60	APEP
ATOM	1623	CA	GLU	204	7.382	29.372	43.414	1.00	17.29	APEP
ATOM	1624	CB	GLU	204	8.093	30.550	42.737	1.00	17.84	APEP
ATOM	1625	CG	GLU	204	9.303	31.084	43.489	1.00	17.21	APEP
ATOM	1626	CD	GLU	204	9.801	32.408	42.937	1.00	17.35	APEP
ATOM	1627	OE1	GLU	204	9.157	32.963	42.023	1.00	15.88	APEP
ATOM	1628	OE2	GLU	204	10.842	32.895	43.422	1.00	17.79	APEP
ATOM	1629	C	GLU	204	6.298	28.842	42.475	1.00	17.89	APEP
ATOM	1630	O	GLU	204	6.383	27.727	41.963	1.00	16.49	APEP
ATOM	1631	N	GLU	205	5.283	29.672	42.251	1.00	19.78	APEP
ATOM	1632	CA	GLU	205	4.159	29.335	41.383	1.00	20.21	APEP
ATOM	1633	CB	GLU	205	2.851	29.854	41.992	1.00	23.42	APEP
ATOM	1634	CG	GLU	205	2.609	31.347	41.772	1.00	28.77	APEP
ATOM	1635	CD	GLU	205	2.874	32.176	43.022	1.00	32.85	APEP
ATOM	1636	OE1	GLU	205	3.942	31.984	43.660	1.00	31.69	APEP
ATOM	1637	OE2	GLU	205	2.008	33.019	43.364	1.00	32.48	APEP
ATOM	1638	C	GLU	205	4.348	29.956	40.009	1.00	18.17	APEP
ATOM	1639	O	GLU	205	4.918	31.039	39.891	1.00	15.97	APEP
ATOM	1640	N	LEU	206	3.863	29.275	38.974	1.00	16.44	APEP
ATOM	1641	CA	LEU	206	3.983	29.785	37.617	1.00	15.66	APEP
ATOM	1642	CB	LEU	206	3.275	28.860	36.626	1.00	15.15	APEP
ATOM	1643	CG	LEU	206	3.869	27.478	36.368	1.00	12.15	APEP
ATOM	1644	CD1	LEU	206	3.189	26.871	35.173	1.00	9.94	APEP
ATOM	1645	CD2	LEU	206	5.370	27.579	36.148	1.00	10.37	APEP
ATOM	1646	C	LEU	206	3.333	31.155	37.561	1.00	15.68	APEP
ATOM	1647	O	LEU	206	3.928	32.125	37.076	1.00	16.18	APEP
ATOM	1648	N	TYR	207	2.105	31.217	38.065	1.00	14.52	APEP
ATOM	1649	CA	TYR	207	1.332	32.451	38.090	1.00	15.05	APEP
ATOM	1650	CB	TYR	207	0.742	32.746	36.705	1.00	12.49	APEP
ATOM	1651	CG	TYR	207	-0.046	31.604	36.083	1.00	12.80	APEP
ATOM	1652	CD1	TYR	207	-1.379	31.365	36.441	1.00	13.16	APEP
ATOM	1653	CE1	TYR	207	-2.113	30.327	35.856	1.00	12.28	APEP
ATOM	1654	CD2	TYR	207	0.533	30.774	35.120	1.00	14.06	APEP
ATOM	1655	CE2	TYR	207	-0.195	29.731	34.528	1.00	14.10	APEP
ATOM	1656	CZ	TYR	207	-1.513	29.515	34.903	1.00	12.49	APEP
ATOM	1657	OH	TYR	207	-2.220	28.487	34.332	1.00	12.47	APEP
ATOM	1658	C	TYR	207	0.206	32.331	39.113	1.00	14.67	APEP
ATOM	1659	O	TYR	207	-0.088	31.244	39.595	1.00	15.57	APEP
ATOM	1660	N	GLN	208	-0.425	33.453	39.432	1.00	15.61	APEP
ATOM	1661	CA	GLN	208	-1.523	33.466	40.393	1.00	15.81	APEP
ATOM	1662	CB	GLN	208	-1.733	34.892	40.896	1.00	14.83	APEP
ATOM	1663	CG	GLN	208	-2.440	34.994	42.231	1.00	16.28	APEP
ATOM	1664	CD	GLN	208	-2.843	36.418	42.564	1.00	16.96	APEP
ATOM	1665	OE1	GLN	208	-2.074	37.364	42.352	1.00	16.63	APEP
ATOM	1666	NE2	GLN	208	-4.051	36.581	43.086	1.00	16.21	APEP
ATOM	1667	C	GLN	208	-2.809	32.947	39.739	1.00	17.01	APEP
ATOM	1668	O	GLN	208	-3.243	33.469	38.717	1.00	14.76	APEP
ATOM	1669	N	THR	209	-3.422	31.921	40.316	1.00	19.39	APEP
ATOM	1670	CA	THR	209	-4.649	31.392	39.736	1.00	23.16	APEP
ATOM	1671	CB	THR	209	-4.792	29.877	39.953	1.00	23.21	APEP
ATOM	1672	OG1	THR	209	-5.010	29.617	41.343	1.00	27.25	APEP
ATOM	1673	CG2	THR	209	-3.559	29.151	39.496	1.00	23.39	APEP
ATOM	1674	C	THR	209	-5.873	32.063	40.340	1.00	25.33	APEP

ATOM	1675	O	THR	209	-5.868	32.455	41.505	1.00	25.28	APEP
ATOM	1676	N	LYS	210	-6.922	32.188	39.536	1.00	27.53	APEP
ATOM	1677	CA	LYS	210	-8.160	32.801	39.986	1.00	29.31	APEP
ATOM	1678	CB	LYS	210	-8.491	34.019	39.122	1.00	30.03	APEP
ATOM	1679	CG	LYS	210	-8.643	33.696	37.647	1.00	28.82	APEP
ATOM	1680	CD	LYS	210	-9.575	34.675	36.963	1.00	29.76	APEP
ATOM	1681	CE	LYS	210	-8.897	35.345	35.771	1.00	28.88	APEP
ATOM	1682	NZ	LYS	210	-9.500	36.669	35.437	1.00	28.04	APEP
ATOM	1683	C	LYS	210	-9.272	31.775	39.873	1.00	31.66	APEP
ATOM	1684	OT1	LYS	210	-10.171	31.775	40.744	1.00	33.57	APEP
ATOM	1685	OT2	LYS	210	-9.224	30.981	38.906	1.00	33.91	APEP
ATOM	1686	OH2	WAT	1001	28.321	31.884	30.023	1.00	4.99	AWAT
ATOM	1687	OH2	WAT	1002	0.070	28.637	38.280	1.00	5.19	AWAT
ATOM	1688	OH2	WAT	1003	9.574	34.984	40.199	1.00	6.03	AWAT
ATOM	1689	OH2	WAT	1004	13.423	28.241	4.674	1.00	6.60	AWAT
ATOM	1690	OH2	WAT	1005	25.593	14.211	8.905	1.00	9.08	AWAT
ATOM	1691	OH2	WAT	1006	-5.948	28.133	30.378	1.00	7.55	AWAT
ATOM	1692	OH2	WAT	1007	13.729	27.746	30.599	1.00	8.15	AWAT
ATOM	1693	OH2	WAT	1008	22.453	33.974	26.365	1.00	6.87	AWAT
ATOM	1694	OH2	WAT	1009	11.644	46.107	27.594	1.00	4.61	AWAT
ATOM	1695	OH2	WAT	1010	-0.650	26.162	33.901	1.00	8.02	AWAT
ATOM	1696	OH2	WAT	1011	8.755	23.060	34.455	1.00	10.12	AWAT
ATOM	1697	OH2	WAT	1012	10.789	39.348	8.288	1.00	3.59	AWAT
ATOM	1698	OH2	WAT	1013	28.091	15.737	14.912	1.00	8.00	AWAT
ATOM	1699	OH2	WAT	1015	16.397	43.678	19.387	1.00	2.04	AWAT
ATOM	1700	OH2	WAT	1016	14.311	29.731	32.127	1.00	5.53	AWAT
ATOM	1701	OH2	WAT	1017	2.570	41.167	21.545	1.00	5.42	AWAT
ATOM	1702	OH2	WAT	1018	25.364	28.506	21.332	1.00	9.41	AWAT
ATOM	1703	OH2	WAT	1019	26.107	50.461	26.214	1.00	5.64	AWAT
ATOM	1704	OH2	WAT	1020	30.469	46.598	34.207	1.00	7.23	AWAT
ATOM	1705	OH2	WAT	1021	30.251	20.969	8.904	1.00	13.93	AWAT
ATOM	1706	OH2	WAT	1022	-4.476	37.486	34.043	1.00	7.76	AWAT
ATOM	1707	OH2	WAT	1023	31.770	27.794	19.020	1.00	13.29	AWAT
ATOM	1708	OH2	WAT	1024	17.644	44.091	30.228	1.00	8.53	AWAT
ATOM	1709	OH2	WAT	1025	-6.207	19.852	35.253	1.00	17.83	AWAT
ATOM	1710	OH2	WAT	1026	14.737	24.657	10.954	1.00	12.71	AWAT
ATOM	1711	OH2	WAT	1027	3.824	43.790	24.674	1.00	11.15	AWAT
ATOM	1712	OH2	WAT	1028	7.499	17.209	26.860	1.00	18.25	AWAT
ATOM	1713	OH2	WAT	1029	0.968	25.199	21.221	1.00	13.34	AWAT
ATOM	1714	OH2	WAT	1030	11.738	36.462	38.807	1.00	14.39	AWAT
ATOM	1715	OH2	WAT	1031	5.648	34.014	38.427	1.00	9.11	AWAT
ATOM	1716	OH2	WAT	1032	1.664	14.320	37.328	1.00	15.77	AWAT
ATOM	1717	OH2	WAT	1033	31.940	28.802	10.755	1.00	8.72	AWAT
ATOM	1718	OH2	WAT	1034	5.832	22.098	18.171	1.00	6.17	AWAT
ATOM	1719	OH2	WAT	1035	33.701	30.509	31.974	1.00	18.85	AWAT
ATOM	1720	OH2	WAT	1036	29.165	34.668	37.418	1.00	10.68	AWAT
ATOM	1721	OH2	WAT	1037	-0.407	43.489	29.418	1.00	8.15	AWAT
ATOM	1722	OH2	WAT	1038	30.861	44.589	26.320	1.00	13.36	AWAT
ATOM	1723	OH2	WAT	1039	8.345	41.081	37.778	1.00	13.31	AWAT
ATOM	1724	OH2	WAT	1040	10.895	22.815	23.399	1.00	20.54	AWAT
ATOM	1725	OH2	WAT	1041	31.503	42.501	27.942	1.00	12.75	AWAT
ATOM	1726	OH2	WAT	1042	-4.123	17.556	26.927	1.00	6.26	AWAT
ATOM	1727	OH2	WAT	1043	23.631	25.350	17.618	1.00	16.68	AWAT
ATOM	1728	OH2	WAT	1044	-9.263	19.789	28.769	1.00	17.07	AWAT
ATOM	1729	OH2	WAT	1045	2.681	26.188	40.094	1.00	10.27	AWAT
ATOM	1730	OH2	WAT	1046	6.157	33.281	40.876	1.00	10.99	AWAT
ATOM	1731	OH2	WAT	1047	1.411	42.305	11.357	1.00	12.65	AWAT

ATOM	1732	OH2	WAT	1048	11.027	43.128	8.836	1.00	13.35	AWAT
ATOM	1733	OH2	WAT	1049	8.163	26.637	9.371	1.00	9.12	AWAT
ATOM	1734	OH2	WAT	1050	30.812	52.897	21.367	1.00	5.26	AWAT
ATOM	1735	OH2	WAT	1051	-1.056	38.906	21.594	1.00	21.26	AWAT
ATOM	1736	OH2	WAT	1052	23.484	37.806	38.523	1.00	5.01	AWAT
ATOM	1737	OH2	WAT	1053	16.091	23.219	9.132	1.00	9.59	AWAT
ATOM	1738	OH2	WAT	1054	10.515	44.724	16.202	1.00	21.22	AWAT
ATOM	1739	OH2	WAT	1055	3.858	42.457	19.188	1.00	18.71	AWAT
ATOM	1740	OH2	WAT	1056	20.767	38.301	29.092	1.00	7.32	AWAT
ATOM	1741	OH2	WAT	1057	31.450	37.717	33.751	1.00	12.78	AWAT
ATOM	1742	OH2	WAT	1058	-6.469	15.556	29.885	1.00	18.83	AWAT
ATOM	1743	OH2	WAT	1059	19.569	32.500	35.567	1.00	13.66	AWAT
ATOM	1744	OH2	WAT	1060	12.883	32.203	45.018	1.00	19.55	AWAT
ATOM	1745	OH2	WAT	1061	16.666	38.811	39.230	1.00	12.36	AWAT
ATOM	1746	OH2	WAT	1062	1.627	24.661	38.597	1.00	11.62	AWAT
ATOM	1747	OH2	WAT	1063	-3.797	23.480	20.462	1.00	13.70	AWAT
ATOM	1748	OH2	WAT	1064	19.662	43.909	20.583	1.00	17.87	AWAT
ATOM	1749	OH2	WAT	1065	28.959	36.788	18.981	1.00	20.15	AWAT
ATOM	1750	OH2	WAT	1066	15.034	47.186	24.909	1.00	7.01	AWAT
ATOM	1751	OH2	WAT	1067	1.479	45.140	16.462	1.00	15.19	AWAT
ATOM	1752	OH2	WAT	1068	-9.159	26.374	32.728	1.00	10.66	AWAT
ATOM	1753	OH2	WAT	1069	18.343	40.026	15.719	1.00	11.74	AWAT
ATOM	1754	OH2	WAT	1070	-4.926	34.883	22.765	1.00	5.57	AWAT
ATOM	1755	OH2	WAT	1071	11.439	44.250	34.445	1.00	17.87	AWAT
ATOM	1756	OH2	WAT	1072	22.346	33.157	38.515	1.00	15.02	AWAT
ATOM	1757	OH2	WAT	1073	16.431	21.026	-0.735	1.00	17.18	AWAT
ATOM	1758	OH2	WAT	1074	17.273	13.097	2.769	1.00	16.44	AWAT
ATOM	1759	OH2	WAT	1075	20.717	41.158	18.875	1.00	11.16	AWAT
ATOM	1760	OH2	WAT	1076	13.429	19.165	10.121	1.00	9.99	AWAT
ATOM	1761	OH2	WAT	1077	22.253	23.110	28.097	1.00	14.11	AWAT
ATOM	1762	OH2	WAT	1078	-1.729	14.634	27.851	1.00	9.23	AWAT
ATOM	1763	OH2	WAT	1079	16.196	36.453	9.936	1.00	18.57	AWAT
ATOM	1764	OH2	WAT	1080	26.774	40.940	39.176	1.00	15.71	AWAT
ATOM	1765	OH2	WAT	1081	27.996	20.266	5.357	1.00	3.12	AWAT
ATOM	1766	OH2	WAT	1082	14.345	44.903	9.828	1.00	16.53	AWAT
ATOM	1767	OH2	WAT	1083	-6.956	19.549	24.667	1.00	13.41	AWAT
ATOM	1768	OH2	WAT	1084	6.677	22.676	16.370	1.00	9.78	AWAT
ATOM	1769	OH2	WAT	1085	24.055	17.307	14.279	1.00	9.70	AWAT
ATOM	1770	OH2	WAT	1086	32.348	29.000	20.500	1.00	15.00	AWAT
ATOM	1771	OH2	WAT	1087	-6.421	34.306	42.856	1.00	13.87	AWAT
ATOM	1772	OH2	WAT	1088	28.806	26.184	27.568	1.00	16.70	AWAT
ATOM	1773	OH2	WAT	1089	9.354	17.475	43.914	1.00	16.99	AWAT
ATOM	1774	OH2	WAT	1090	30.672	20.876	12.987	1.00	21.44	AWAT
ATOM	1775	OH2	WAT	1091	-7.795	23.654	35.198	1.00	12.77	AWAT
ATOM	1776	OH2	WAT	1092	6.675	42.663	7.635	1.00	17.23	AWAT
ATOM	1777	OH2	WAT	1093	14.348	49.247	34.229	1.00	10.41	AWAT
ATOM	1778	OH2	WAT	1094	-2.481	40.065	24.802	1.00	16.54	AWAT
ATOM	1779	OH2	WAT	1095	-5.184	39.229	18.838	1.00	28.58	AWAT
ATOM	1780	OH2	WAT	1096	-6.282	29.165	17.208	1.00	27.91	AWAT
ATOM	1781	OH2	WAT	1097	3.526	19.041	19.713	1.00	16.33	AWAT
ATOM	1782	OH2	WAT	1098	-5.490	40.336	27.666	1.00	20.30	AWAT
ATOM	1783	OH2	WAT	1099	5.791	43.554	30.434	1.00	14.00	AWAT
ATOM	1784	OH2	WAT	1100	10.085	34.242	9.352	1.00	19.88	AWAT
ATOM	1785	OH2	WAT	1101	22.752	37.487	8.325	1.00	16.96	AWAT
ATOM	1786	OH2	WAT	1102	22.364	40.020	38.129	1.00	15.64	AWAT
ATOM	1787	OH2	WAT	1103	33.666	37.537	19.756	1.00	23.15	AWAT
ATOM	1788	OH2	WAT	1104	36.579	34.638	23.256	1.00	16.03	AWAT

ATOM	1789	OH2	WAT	1105	31.645	31.136	11.971	1.00	18.60	AWAT
ATOM	1790	OH2	WAT	1106	14.823	26.519	41.694	1.00	15.23	AWAT
ATOM	1791	OH2	WAT	1107	13.638	21.317	9.375	1.00	12.16	AWAT
ATOM	1792	OH2	WAT	1108	33.913	48.939	32.690	1.00	11.76	AWAT
ATOM	1793	OH2	WAT	1109	33.415	48.326	34.490	1.00	20.71	AWAT
ATOM	1794	OH2	WAT	1110	-8.560	41.287	43.725	1.00	12.63	AWAT
ATOM	1795	OH2	WAT	1111	22.656	24.209	0.884	1.00	17.46	AWAT
ATOM	1796	OH2	WAT	1112	2.716	41.252	15.063	1.00	19.18	AWAT
ATOM	1797	OH2	WAT	1113	30.635	31.007	7.714	1.00	10.21	AWAT
ATOM	1798	OH2	WAT	1114	14.815	22.010	39.023	1.00	27.49	AWAT
ATOM	1799	OH2	WAT	1115	33.286	47.303	24.007	1.00	12.66	AWAT
ATOM	1800	OH2	WAT	1116	14.042	33.412	10.622	1.00	10.91	AWAT
ATOM	1801	OH2	WAT	1117	20.195	27.499	32.658	1.00	18.38	AWAT
ATOM	1802	OH2	WAT	1118	31.215	17.825	13.678	1.00	17.20	AWAT
ATOM	1803	OH2	WAT	1119	30.831	21.030	11.086	1.00	17.97	AWAT
ATOM	1804	OH2	WAT	1120	30.910	27.311	25.284	1.00	17.48	AWAT
ATOM	1805	OH2	WAT	1121	6.259	13.355	26.082	1.00	21.34	AWAT
ATOM	1806	OH2	WAT	1122	34.780	29.659	15.089	1.00	23.24	AWAT
ATOM	1807	OH2	WAT	1123	33.170	28.242	23.488	1.00	16.58	AWAT
ATOM	1808	OH2	WAT	1124	0.913	40.672	41.455	1.00	17.75	AWAT
ATOM	1809	OH2	WAT	1125	25.393	36.689	42.266	1.00	22.18	AWAT
ATOM	1810	OH2	WAT	1126	21.923	40.748	15.035	1.00	20.08	AWAT
ATOM	1811	OH2	WAT	1127	-1.339	28.433	21.094	1.00	17.33	AWAT
ATOM	1812	OH2	WAT	1128	22.058	28.769	33.380	1.00	22.93	AWAT
ATOM	1813	OH2	WAT	1129	2.232	23.035	17.663	1.00	13.73	AWAT
ATOM	1814	OH2	WAT	1130	4.834	40.228	40.117	1.00	39.84	AWAT
ATOM	1815	OH2	WAT	1131	16.182	27.937	1.692	1.00	9.10	AWAT
ATOM	1816	OH2	WAT	1132	36.696	43.322	33.662	1.00	14.41	AWAT
ATOM	1817	NA	NAT	500	-4.312	15.332	28.374	1.00	11.67	ANAT

END

The solvent accessibilities of Ves v 5 amino acid residues are given in Table 7.

Table 7

Surface Exposure of Ves v 5 amino acids

NO	AA	Solv exp
3	E	0.802
4	A	0.060
5	E	0.390
6	F	0.868
7	N	0.484
8	N	0.555
9	Y	0.033
10	C	0.412
11	K	0.978
12	I	0.225
13	K	0.951
14	C	0.038
15	L	0.714
16	K	1.000
17	G	0.143

18	G	0.275
19	V	0.445
20	H	0.016
21	T	0.000
22	A	0.049
23	C	0.209
24	K	0.489
25	Y	0.280
26	G	0.352
27	S	0.159
28	L	0.423
29	K	0.797
30	P	0.231
31	N	0.396
32	C	0.055
33	G	0.429
34	N	0.775
35	K	0.297
36	V	0.489
37	V	0.280
38	V	0.379
39	S	0.291
40	Y	0.593
41	G	0.165
42	L	0.121
43	T	0.423
44	K	0.978
45	Q	0.538
46	E	0.264
47	K	0.396
48	Q	0.593
49	D	0.302
50	I	0.000
51	L	0.198
52	K	0.615
53	E	0.170
54	H	0.000
55	N	0.115
56	D	0.445
57	F	0.027
58	R	0.000
59	Q	0.198
60	K	0.407
61	I	0.000
62	A	0.033
63	R	0.956
64	G	0.148
65	L	0.593
66	E	0.005
67	T	0.610

68	R	0.335
69	G	0.110
70	N	0.549
71	P	0.363
72	G	0.170
73	P	0.440
74	Q	0.005
75	P	0.209
76	P	0.236
77	A	0.022
78	K	0.775
79	N	0.236
80	M	0.066
81	K	0.588
82	N	0.500
83	L	0.016
84	V	0.462
85	W	0.275
86	N	0.165
87	D	0.621
88	E	0.247
89	L	0.005
90	A	0.055
91	Y	0.115
92	V	0.005
93	A	0.000
94	Q	0.159
95	V	0.011
96	W	0.077
97	A	0.000
98	N	0.005
99	Q	0.027
100	C	0.027
101	Q	0.577
102	Y	0.687
103	G	0.110
104	H	0.549
105	D	0.022
106	T	0.429
107	C	0.000
108	R	0.203
109	D	0.093
110	V	0.044
111	A	0.500
112	K	0.824
113	Y	0.209
114	Q	0.423
115	V	0.011
116	G	0.011
117	Q	0.066

118	N	0.005
119	V	0.022
120	A	0.016
121	L	0.198
122	T	0.198
123	G	0.231
124	S	0.236
125	T	0.610
126	A	0.253
127	A	0.379
128	K	0.857
129	Y	0.352
130	D	0.220
131	D	0.495
132	P	0.033
133	V	0.137
134	K	0.654
135	L	0.000
136	V	0.000
137	K	0.538
138	M	0.473
139	W	0.016
140	E	0.071
141	D	0.341
142	E	0.154
143	V	0.000
144	K	0.560
145	D	0.390
146	Y	0.044
147	N	0.165
148	P	0.214
149	K	0.868
150	K	0.604
151	K	0.753
152	F	0.071
153	S	0.302
154	G	0.192
155	N	0.121
156	D	0.379
157	F	0.819
158	L	0.714
159	K	0.533
160	T	0.000
161	G	0.077
162	H	0.231
163	Y	0.000
164	T	0.000
165	Q	0.011
166	M	0.000
167	V	0.000

168	W	0.005
169	A	0.011
170	N	0.429
171	T	0.000
172	K	0.451
173	E	0.165
174	V	0.000
175	G	0.000
176	C	0.016
177	G	0.000
178	S	0.016
179	I	0.000
180	K	0.214
181	Y	0.016
182	I	0.275
183	Q	0.231
184	E	0.841
185	K	0.989
186	W	0.665
187	H	0.159
188	K	0.203
189	H	0.011
190	Y	0.000
191	L	0.000
192	V	0.000
193	C	0.000
194	N	0.000
195	Y	0.000
196	G	0.000
197	P	0.225
198	S	0.110
199	G	0.027
200	N	0.308
201	F	0.341
202	K	0.824
203	N	0.797
204	E	0.374
205	E	0.511
206	L	0.055
207	Y	0.082
208	Q	0.566
209	T	0.473
210	K	0.962

Example 10 Alignment of Ag 5s

An alignment of selected antigen 5 sequences from Vespula, Dolichovespula , Vespa, Polistes and Solenopsis (fire ants) is shown in Fig. 12. Vespula, Dolichovespula, Vespa and Polistes all belong to the Vespidae family. The figure also includes the secondary

structural elements of Ves v 5. When considering only the Vespula antigen 5s a very high degree of surface conservation is observed (Fig. 5), the conservation of residues being almost evenly distributed with only a few non-conserved residues scattered over the molecule.

In contrast, the surfaces conserved, when comparing sequences from the Vespula and Polistes genera, are restricted to 5 regions with solvent accessible areas of 392 Å^2, 585 Å^2, 589 Å^2, 673 Å^2 and 1053 Å^2, respectively. Solvent accessibility was calculated using the NACCESS program (S. J. Hubbard and J. M. Thornton, 1992, NACCESS. (v2.1.1) Department of Biochemistry and Molecular Biology, University College London) with a probe radius of 1.4 Å. Similarly, five surface patches corresponding to the 5 surface patches conserved between Vespula and Polistes, were conserved between Vespula and Vespa/Dolichovespula. In the latter case the areas are 280 Å^2, 496 Å^2, 730 Å^2, 803 Å^2 and 1043 Å^2, respectively. The residues contributing to one surface patch are primarily from the beginning of the B strand and from helix IV, the residues contributing to a second surface patch are primarily from the A strand and the loop between helix II and strand B, the residues contributing to a third surface patch is primarily from helix I and its surroundings and from the end of helix II, the residues contributing to a fourth surface patch is mainly of N-terminal origin while a fifth surface patch is dominated by residues from the end of helix I and the loop between helix I and the A strand.

DISCUSSION

Crystallographic studies of protein antigen-antibody complexes have shown that the contact residues of an epitope may contain as many as 17 residues on the surface of an antigen, and that these residues may, or may not, be contiguous to each other in the peptide chain (Davies *et al.*, 1996, Proc. Natl Acad. Sci USA, 93:7). Epitope mapping of lysozyme with monoclonal antibodies have shown that the entire surface of a protein is potentially antigenic (Newmann *et al.*, 1992, J. Immunol. 149:3260). Thus the hybrids with 1/10 to 3/4 of yellow jacket antigen 5, will have fewer epitopes than the parent molecule.

The CD spectral data in Figure 7 suggest that the hybrids have secondary structures closely similar, if not identical, with those of vespid antigen 5s. The inhibition data in Figures 8 and 9 with Ves v 5-specific human and mouse antibodies and the antibody binding data in Table 3 with hybrid-specific antibodies suggest that the hybrids have tertiary

structures closely similar or identical with that of Ves v 5, as these antibodies do not bind the denatured Ves v 5. Additional evidence came from screening with 17 monoclonal mouse IgG1 antibodies specific for the natural Ves v 5, six of which bound the N-terminal hybrid PV1-46. Therefore these data indicate that the hybrids contain the discontinuous B cell epitopes of Ves v 5.

The inhibition data with polyclonal antibodies and the binding data with monoclonal antibodies indicate that the dominant B cell epitopes of Ves v 5 are in its N-terminal region. Inspection of the structure of Ves v 5 in shows that nearly all residues in the N-terminal hybrid PV1-46 are surface accessible. (See Table 7) This is in contrast to the C-terminal hybrid PV156-204, in which only segments of Ves v 5 are surface accessible. (See Table 7) This difference in surface accessibility may explain the immunodominance of the N-terminal region of antigen 5. Others have shown that the entire surface of a protein is potentially antigenic but the regions with high surface accessibility and surface protrusion are dominant (Newmann *et al.*, 1992, J. Immunol 149:3260 and Novotny et al., 1996, Adv Prot Chem 49:149).

At present the only known way to map discontinuous epitopes is by X-ray crystallography of Ag-Ab complexes (Davies *et al.*, 1996, Proc. Natl Acad. Sci USA, 93:7) and this requires having specific monoclonal antibodies. The discontinuous epitopes of CD39 was mapped with a series of mouse-human hybrids, mouse and human CD39 molecules have 75% sequence identity and they share limited antigenic cross-reactivity (Maliszewski *et al.*, 1994, J. Immunol 153:3574). These findings with CD39 and antigen 5 indicate that hybrids of two homologous proteins represent a useful approach to mapping their discontinuous B cell epitopes.

Our results with hybrid Ag 5s demonstrate that hybrid allergens can have a hundred to a thousand-fold reduction in allergenicity yet retain the immunogenicity of the natural allergens. This reduction in allergenicity of hybrids is believed to be mainly due to a decrease of B cell epitope density. Each hybrid of the Examples has only a portion of the B and T cell epitopes of Ves v 5. In principle, however, a mixture of hybrids can reconstitute the complete epitope library of Ves v 5. Thus, all epitopes can be reconstituted to prepare modified allergens for use as vaccines. Our results suggest that a PV hybrid with 20-30

residues of Ves v 5 will have maximal reduction in allergenicity yet retaining immunogenicity for Ves v 5.

Many allergens have sequence homology with proteins from diverse sources (Larsen *et al.*, 1996, J Allergy Clin Immunol 97:577). For example, vespid Ag 5s have varying degrees of sequence homology with a variety of extracellular proteins from different organisms, ranging from fungi to humans (see Fig. 12). It is known that homologous proteins of 30% sequence identity may have the same or closely similar structures (Chothia *et al.*, 1990, Annual Review Biochem 59:1007 and Russell *et al.*, 1994, J. Mol. Biol. 244:332). Thus, hybrids may be prepared with a variety of homologous host proteins to function as scaffolds for the guest allergen fragment of interest.

* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference.

Table 8. Allergens

ORGANISM	ALLERGEN	PROTEIN	SIZE (kD)	C/P[a]	REFERENCE/ ACCESSION NO
Weed pollens					
Asterales					
Ambrosia artemisiifolia short ragweed	Amb a 1 Amb a 2 Amb a 3 Amb a 5 Amb a 6 Amb a 7 Amb a ?	antigen E antigen K Ra3 Ra5 Ra6 Ra7	38 38 11 5 10 12 11	C C C C P C C	8, 20 8, 21 22 11, 23 24, 25 26 27
Ambrosia trifida giant ragweed	Amb t 5	Ra5G	4.4	C	9, 10, 28
Artemisia vulgaris mugwort	Art v 1 Art v 2 Art v 3 Art v 4		27-29 35 12 14	C P P C	28A 29 53
Helianthus annuus sunflower	Hel a 1 Hel a 2	 profilin	34 15.7	 C	29A Y15210
Mercurialis annua	Mer a 1	profilin	14-15	C	Y13271
Caryophyllales					
Salsola kali Russian thistle	Sal k 1	43	43	P	29B
Grass pollens					
Poales					
Cynodon dactylon Bermuda grass	Cyn d 1 Cyn d 7 Cyn d 12	 profilin	32 14	C C C	30, S83343 31, X91256 31a, Y08390

Dactylis glomerata orchard grass	Dac g 1 Dac g 2 Dac g 3 Dac g 5	AgDg1	32 11 31	P C C P	32 33, S45354 33A, U25343 34
Holcus lanatus velvet grass	Hol l 1			C	Z27084
Lolium perenne rye grass	Lol p 1 Lol p 2 Lol p 3 Lol p 5 Lol p 11	group I group II group III Lol p IX, Lol p Ib hom: trypsin inhibitor	27 11 11 31/35 16	C P P C	35, 36 37, 37A, X73363 38 34, 39 39A
Phalaris aquatica canary grass	Pha a 1			C	40, S80654
Phleum pratense timothy	Phl p 1 Phl p 2 Phl p 4 Phl p 5 Phl p 6 Phl p 12 Phl p 13	 Ag25 profilin polygalacturonase	27 32 55-60	C C P C C C C	X78813 41, X75925 41A 42 43, Z27082 44, X77583 AJ238848
Poa pratensis Kentucky blue grass	Poa p 1 Poa p 5	group I	33 31/34	P C	46 34, 47
Sorghum halepense Johnson grass	Sor h 1			C	48
Tree pollens					
Fagales					
Alnus glutinosa alder	Aln g 1		17	C	S50892
Betula verrucosa birch	Bet v 1 Bet v 2 Bet v 3 Bet v 4 Bet v 6 Bet v 7	 profilin h: isoflavone reductase cyclophilin	17 15 8 33.5 18	C C C C C P	49,50, Z80098 M65179 X79267 X87153, S54819 AF135127 P81531
Carpinus betulus hornbeam	Car b 1		17	C	51, X66932, X66918

Castanea sativa chestnut	Cas s 1		22	P	52
	Cas s 5	chitinase			
	Cas s 8	lipid transfer protein	9.7	p	53
Corylus avellana hazel	Cor a 1		17	C	54A, X70999
	Cor a 2	profilin	14	C	AF327622
Quercus alba White oak	Que a 1		17	P	54
Lamiales					
Oleaceae					
Fraxinus excelsior ash	Fra e 1		20	P	58A
Ligustrum vulgare privet	Lig v 1		20	P	58A
Olea europea olive	Ole e 1		16	C	59, 60
	Ole e 2	profilin	15-18	C	60A
	Ole e 3		9.2		60B
	Ole e 4		32	P	P80741
	Ole e 5	superoxide dismutase	16	P	P80740
	Ole e 6		10	C	60C, U86342
	Ole e 7			P	60D, P81430
Syringa vulgaris lilac	Syr v 1		20	P	58A
Plantaginaceae					
Plantago lanceolata English plantain	Pla l 1		18	P	P842242
Pinales					
Cryptomeria japonica sugi	Cry j 1		41-45	C	55, 56
	Cry j 2			C	57, D29772
Cupressus arizonica cypress	Cup a 1		43	C	A1243570
Juniperus ashei mountain cedar	Jun a 1		43	P	P81294
	Jun a 2	-		C	57A, AJ404653
	Jun a 3		30	P	57B, P81295
Juniperus oxycedrus prickly juniper	Jun o 4	hom: calmodulin	29	C	57C, AF031471

Juniperus sabinoides mountain cedar	Jun s		50	P	58
Juniperus virginiana eastern red cedar	Jun v 1		43	P	P81825
Mites					
Acarus siro mite	Aca s 13	fatty acid binding prot	14*	C	AJ006774
Blomia tropicalis mite	Blo t 5 Blo t 12 Blo t 13	 Bt11a Bt6, fatty acid bind prot.		C C C	U59102 U27479 U58106
Dermatophagoides pteronyssinus mite	Der p 1 Der p 2 Der p 3 Der p 4 Der p 5 Der p 6 Der p 7 Der p 8 Der p 9 Der p 10 Der p 14	antigen P1 trypsin amylase chymotrypsin glutathione transferase collagenolytic serine pro. tropomyosin apolipophorin like prot.	25 14 28/30 60 14 25 22/28 36 	C C C P C P C C P C C	61 62 63 64 65 66 67 67A 67B Y14906
Dermatophagoides microceras mite	Der m 1		25	P	68
Dermatophagoides farinae mite	Der f 1 Der f 2 Der f 3 Der f 10 Der f 11 Der f 14 Derf f 15 Derf f 16 Derf f 17	 tropomyosin paramyosin mag3, apolipophorin 98k chitinase gelsolin/villin Ca binding EF protein	25 14 30 98 98 53 53	C C C C C C C C C	69 70, 71 63 72 72A D17686 AF178772 71A 71A
Euroglyphus maynei mite	Eur m 14	apolipophorin	177	C	AF149827

Lepidoglyphus destructor storage mite	Lep d 2		15	C	73, 74, 75
	Lep d 5			C	75A, AJ 250278
	Lep d 7			C	75A, AJ271058
	Lep d 10	tropomyosin		C	AJ25096
	Lep d 13			C	75A, AJ250279
Animals					
Bos domesticus domestic cattle (see also foods)	Bos d 2	Ag3, lipocalin	20	C	76, L42867
	Bos d 3	Ca-binding S100 hom	11	C	L39834
	Bos d 4	alpha-lactalbumin	14.2	C	M18780
	Bos d 5	beta-lactoglobulin	18.3	C	X14712
	Bos d 6	serum albumin	67	C	M73993
	Bos d 7	immunoglobulin	160		77
	Bos d 8	caseins	20-30		77
Canis familiaris (Canis domesticus) dog	Can f 1		25	C	78, 79
	Can f 2		27	C	78, 79
	Can f 3	albumin		C	S72946
Equus caballus domestic horse	Equ c 1	lipocalin	25	C	U70823
	Equ c 2	lipocalin	18	P	79A, 79B
	Equ c 3	Ag3-X	67	C	79C,X74045
	Equ c 4		17	P	79D
	Equ c 5	AgX	17	P	
Felis domesticus cat (saliva)	Fel d 1	cat-1	38	C	15
	Fel d 2	albumin		C	79E, X84842
	Fel d 3	cystatin	11	C	79F, AF238996
Mus musculus mouse (urine)	Mus m 1	MUP	19	C	80, 81
Rattus norvegius rat (urine)	Rat n 1		17	C	82, 83
Fungi (moulds)					
Ascomycota					
Dothidiales					

Alternaria alternata	Alt a 1		28	C	U82633
	Alt a 2		25	C	83A, U62442
	Alt a 3	heat shock prot. 70		C	U87807, U87808
	Alt a 4	prot. disulfideisomerase	57	C	X84217
	Alt a 6	acid ribosomal prot. P2	11	C	X78222, U87806
	Alt a 7	YCP4 protein	22	C	X78225
	Alt a 10	aldehyde dehydrogenase	53	C	X78227, P42041
	Alt a 11	enolase	45	C	U82437
	Alt a 12	acid ribosomal prot. P1	11	C	X84216
Cladosporium herbarum	Cla h 1		13		83B, 83C
	Cla h 2		23		83B, 83C
	Cla h 3	aldehyde dehydrogenase	53	C	X78228
	Cla h 4	acid ribosomal prot. P2	11	C	X78223
	Cla h 5	YCP4 protein	22	C	X78224
	Cla h 6	enolase	46	C	X78226
	Cla h 12	acid ribosomal prot. P1	11	C	X85180
Eurotiales					
Aspergillus flavus	Asp fl 13	alkaline serine protease	34		84
Aspergillus fumigatus	Asp f 1		18	C	M83781, S39330
	Asp f 2		37	C	U56938
	Asp f 3	peroxisomal protein	19	C	U20722
	Asp f 4		30	C	AJ001732
	Asp f 5	metalloprotease	40	C	Z30424
	Asp f 6	Mn superoxide dismut.	26.5	C	U53561
	Asp f 7		12	C	AJ223315
	Asp f 8	ribosomal prot. P2	11	C	AJ224333
	Asp f 9		34	C	AJ223327
	Asp f 10	aspartic protease	34	C	X85092
	Asp f 11	peptidyl-prolyl isomeras	24		84A
	Asp f 12	heat shock prot. P90	90	C	85
	Asp f 13	alkaline serine protease	34		84B
	Asp f 15		16	C	AJ002026
	Asp f 16		43	C	g3643813
	Asp f 17			C	AJ224865
	Asp f 18	vacuolar serine protease	34		84C
Aspergillus niger	Asp n 14	beta-xylosidase	105	C	AF108944
	Asp n 18	vacuolar serine protease	34	C	84B
	Asp n ?		85	C	Z84377
Aspergillus oryzae	Asp o 13	alkaline serine protease	34	C	X17561
	Asp o 21	TAKA-amylase A	53	C	D00434, M33218
Penicillium brevicompactum	Pen b 13	alkaline serine protease	33		86A

Penicillium citrinum	Pen c 3 Pen c 13 Pen c 19 Pen c 22w	peroxisomal mem. prot. alkaline serine protease heat shock prot. P70 enolase	18 33 70 46	 C C	86B 86A U64207 AF254643
Penicillium notatum	Pen n 13 Pen n 18 Pen n 20	alkaline serine protease vacuolar serine protease N-acetyl glucosaminidas	34 32 68		89 89 87
Penicillium oxalicum	Pen o 18	vacuolar serine protease	34		89
Onygenales					
Trichophyton rubrum	Tri r 2 Tri r 4	 serine protease		C C	90 90
Trichophyton tonsurans	Tri t 1 Tri t 4	 serine protease	30 83	P C	91 90
Saccharomycetales					
Candida albicans	Cand a 1		40	C	88
Candida boidinii	Cand b 2		20	C	J04984, J04985
Basidiomycota					
Basidiolelastomycetes					
Malassezia furfur	Mala f 1 Mala f 2 Mala f 3 Mala f 4 Mala f 5 Mala f 6	 MF1, peroxisomal membrane protein MF2, peroxisomal membrane protein	 21 20 35 18* 17*	 C C C C C	91A AB011804 AB011805 AJ011955 AJ011956
Basidiomycetes					
Psilocybe cubensis	Psi c 1 Psi c 2	 cyclophilin	 16		 91B
Coprinus comatus shaggy cap	 Cop c 1 Cop c 2 Cop c 3 Cop c 5 Cop c 7	 leucine zipper protein	 11	 C	 AJ132235 AJ242791 AJ242792 AJ242793 AJ242794
Insects					
Aedes aegyptii mosquito	 Aed a 1 Aed a 2	 apyrase	 68 37	 C C	 L12389 M33157

Apis mellifera honey bee	Api m 1	phospholipase A2	16	C	92
	Api m 2	hyaluronidase	44	C	93
	Api m 4	melittin	3	C	94
	Api m 6		7-8	P	
Bombus pennsylvanicus bumble bee	Bom p 1	phospholipase	16	P	95
	Bom p 4	protease		P	95
Blattella germanica German cockroach	Bla g 1	Bd90k		C	
	Bla g 2	aspartic protease	36	C	96
	Bla g 4	calycin	21	C	97
	Bla g 5	glutathione transferase	22	C	98
	Bla g 6	troponin C	27	C	98
Periplaneta americana American cockroach	Per a 1	Cr-PII		C	
	Per a 3	Cr-PI	72-78	C	98A
	Per a 7	tropomyosin	37	C	Y14854

Chironomus thummi thummi midges	Chi t 1-9 Chi t 1.01 Chi t 1.02 Chi t 2.0101 Chi t 2.0102 Chi t 3 Chi t 4 Chi t 5 Chi t 6.01 Chi t 6.02 Chi t 7 Chi t 8 Chi t 9	hemoglobin component III component IV component I component IA component II-beta component IIIA component VI component VIIA component IX component VIIB component VIII component X	16 16 16 16 16 16 16 16 16 16 16 16 16	C C C C C C C C C C C C C	99 P02229 P02230 P02221 P02221 P02222 P02231 P02224 P02226 P02223 P02225 P02227 P02228
Dolichovespula maculata white face hornet	Dol m 1 Dol m 2 Dol m 5	phospholipase A1 hyaluronidase antigen 5	35 44 23	C C C	100 101 102, 103
Dolichovespula arenaria yellow hornet	Dol a 5	antigen 5	23	C	104
Polistes annularies wasp	Pol a 1 Pol a 2 Pol a 5	phospholipase A1 hyaluronidase antigen 5	35 44 23	P P C	105 105 104
Polistes dominulus Mediterranean paper wasp	Pol d 1 Pol d 4 Pol d 5	serine protease	32-34	C	P81656
Polistes exclamans wasp	Pol e 1 Pol e 5	phospholipase A1 antigen 5	34 23	P C	107 104
Polistes fuscatus wasp	Pol f 5	antigen 5	23	C	106
Polistes metricus wasp	Pol m 5	antigen 5	23	C	106
Vespa crabo European hornet	Vesp c 1 Vesp c 5	phospholipase antigen 5	34 23	P C	107 106
Vespa mandarina giant asian hornet	Vesp m 1 Vesp m 5				P81657
Vespula flavopilosa yellowjacket	Ves f 5	antigen 5	23	C	106

Vespula germanica yellowjacket	Ves g 5	antigen 5	23	C	106
Vespula maculifrons yellowjacket	Ves m 1 Ves m 2 Ves m 5	phospholipase A1 hyaluronidase antigen 5	33.5 44 23	C P C	108 109 104
Vespula pennsylvanica yellowjacket	Ves p 5	antigen 5	23	C	106
Vespula squamosa yellowjacket	Ves s 5	antigen 5	23	C	106
Vespula vidua wasp	Ves vi 5	antigen 5	23	C	106
Vespula vulgaris yellowjacket	Ves v 1 Ves v 2 Ves v 5	phospholipase A1 hyaluronidase antigen 5	35 44 23	C P C	105A 105A 104
Myrmecia pilosula Australian jumper ant	Myr p 1 Myr p 2			C C	X70256 S81785
Solenopsis geminata tropical fire ant	Sol g 2 Sol g 4				
Solenopsis invicta fire ant	Sol i 2 Sol i 3 Sol i 4		13 24 13	C C C	110, 111 110 110
Solenopsis saevissima Brazilian fire ant	Sol s 2				
Foods					
Gadus callarias cod	Gad c 1	allergen M	12	C	112, 113
Salmo salar Atlantic salmon	Sal s 1	parvalbumin	12	C	X97824
Bos domesticus domestic cattle (milk)	Bos d 4 Bos d 5 Bos d 6 Bos d 7 Bos d 8	alpha-lactalbumin beta-lactoglobulin serum albumin immunoglobulin caseins	14.2 18.3 67 160 20-30	C C C	M18780 X14712 M73993 77 77

Gallus domesticus chicken	Gal d 1 Gal d 2 Gal d 3 Gal d 4 Gal d 5	ovomucoid ovalbumin Ag22, conalbumin lysozyme serum albumin	28 44 78 14 69	C C C C C	114, 115 114, 115 114, 115 114, 115 X60688
Metapenaeus ensis shrimp	Met e 1	tropomyosin	C		U08008
Penaeus aztecus shrimp	Pen a 1	tropomyosin	36	P	116
Penaeus indicus shrimp	Pen i 1	tropomyosin	34	C	117
Todarodes pacificus squid	Tod p 1	tropomyosin	38	P	117A
Haliotis midae abalone	Hal m 1		49		117B
Apium graveolens celery	Api g 1 Api g 4 Api g 5	hom: Bet v 1 profilin	16* 55/58	C P	Z48967 AF129423 P81943
Brassica juncea oriental mustard	Bra j 1	2S albumin	14	C	118
Brassica rapa turnip	Bra r 2	hom: prohevein	25		P81729
Hordeum vulgare barley	Hor v 15	BMAI-1	15	C	119
Zea mays maize, corn	Zea m 14	lipid transfer protein	9	P	P19656
Oryza sativa rice	Ory s 1			C	U31771
Corylus avellana hazelnut	Cor a 1.0401	hom: Bet v 1	17	C	AF136945
Malus domestica apple	Mal d 1 Mal d 2 Mal d 3	hom: Bet v 1 hom: thaumatin lipid transfer protein	 9	C C C	X83672 AJ243427
Pyrus communis pear	Pyr c 1 Pyr c 4 Pyr c 5	hom: Bet v 1 profilin hom: isoflavone reductas	18 14 33.5	C C C	AF05730 AF129424 AF071477

Persea americana avocado	Pers a 1	endochitinase	32	C	Z78202
Prunus armeniaca apricot	Pru ar 1 Pru ar 3	hom: Bet v 1 lipid transfer protein	 9	C P	U93165
Prunus avium sweet cherry	Pru av 1 Pru av 2 Pru av 3 Pru av 4	hom: Bet v 1 hom: thaumatin lipid transfere protein profilin	 10 15	C C C C	U66076 U32440 AF221501 AF129425
Prunus domestica European plum	Pru d 3	lipid transfer protein	9	P	119A
Prunus persica peach	Pru p 3	lipid transfer protein	10	P	P81402
Vitis vinifera grape	Vit v 1	lipid transfer protein	9	P	P80274
Musa x paradisiaca banana	Mus xp 1	profilin	15	C	AF377948
Ananas comosus pineapple	Ana c 1	profilin	15	C	AF377949
Lichti chinensis litchi	Lit c 1	profilin	15	C	AY049013
Sinapis alba yellow mustard	Sin a 1	2S albumin	14	C	120
Glycine max soybean	Gly m 1 Gly m 2 Gly m 3	HPS profilin	7 8 14	P P C	121 A57106 AJ223982
Arachis hypogaea peanut	Ara h 1 Ara h 2 Ara h 3 Ara h 4 Ara h 5 Ara h 6 Ara h 7	vicilin conglutin glycinin glycinin profilin hom: conglutin hom: conglutin	63.5 17 60 37 15 15 15	C C C C C C C	L34402 L77197 AF093541 AF086821 AF059616 AF092846 AF091737
Actinidia chinensis kiwi	Act c 1	cysteine protease	30	P	P00785
Capsicum annum bell pepper	Cap a 1w	osmotin-like protein	23	c	AJ297410

Solanum tuberosum potato	Sola t 1 Sola t 2 Sola t 3 Sola t 4	patatin cathepsin D inhibitor cys. protease inhibitor asp. protease inhibitor	43 21 21 16+4	P P P P	P15476 P16348 P20347 P30941
Bertholletia excelsa Brazil nut	Ber e 1	2S albumin	9	C	P04403, M17146
Juglans regia English walnut	Jug r 1 Jug r 2	2S albumin vicilin	 44	C C	U66866 AF066055
Ricinus communis Castor bean	Ric c 1	2S albumin		C	P01089
Sesamum indicum sesame	Ses i 1 Ses i 2 Ses i 3	2S albumin 2S albumin 7S vicilin-like globulin	9 7 45	C C C	121A, AF240005 AF091841 AF240006
Cucumis melo muskmelon	Cuc m 1	serine protease	66	C	D32206
Additional:					
Anisakis simplex nematode	Ani s 1 Ani s 2 Ani s 3	 paramyosin tropomyosin	24 97 41	P C C	121B, A59069 AF173004 121C, Y19221
Ascaris suum worm	Asc s 1		10	P	122
Dendronephthya nipponica soft coral	Den n 1		53	P	122A

Hevea brasiliensis rubber (latex)					
	Hev b 1	elongation factor	58	P	123, 124
	Hev b 2	1,3-glucanase	34/36	C	125
	Hev b 3		24	P	126, 127
	Hev b 4	component of microhelix complex	100-115	P	128
	Hev b 5		16	C	U42640
	Hev b 6.01	hevein precursor	20	C	M36986, p02877
	Hev b 6.02	hevein	5	C	M36986, p02877
	Hev b 6.03	C-terminal fragment	14	C	M36986, p02877
	Hev b 7.01	hom: patatin from B-serum	42	C	U80598
	Hev b 7.02	hom: patatin from C-serum	44	C	AJ223038
	Hev b 8	profilin	14	C	Y15042, AJ132397, AF119365, AF119366
	Hev b 9	enolase	51	C	AJ132580
	Hev b 10	Mn superoxide dismut.	26	C	AJ249148
	Hev b 11w	class 1 chitinase		C	AJ238579
	Hev b 12	lipid transfer protein	9.3	C	
Ctenocephalides felis felis cat flea					
	Cte f 1				
	Cte f 2	M1b	27	C	AF231352
Homo sapiens human autoallergens					
	Hom s 1		73*	C	Y14314
	Hom s 2		10.3*	C	X80909
	Hom s 3		20.1*	C	X89985
	Hom s 4		36*	C	Y17711
	Hom s 5		42.6*	C	P02538

[a]Clone (C) or Protein (P) data

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Table 9. Selected allergens with structures available in Protein Database (PDB)

ID NO: 1A0K

Deposited: 02-Dec-1997 Exp. Method: X-ray Diffraction Resolution: 2.20 Å

Title	Profilin I From Arabidopsis Thaliana
Classification	Cytoskeleton
Compound	Mol_Id: 1; Molecule: Profilin; Chain: Null; Engineered: Recombinant Plant Protein; Biological_Unit: Monomer

ID NO: 1A9V

Deposited: 10-Apr-1998 Exp. Method: NMR, 10 Structures

Title	Tertiary Structure Of The Major House Dust Mite Allergen Der P 2, NMR, 10 Structures
Classification	Allergen
Compound	Mol_Id: 1; Molecule: Mite Allergen Der P 2; Chain: Null; Engineered: Yes; Mutation: D1S; Other_Details:D1S Mutant Made To Enhance N-Terminal Met Removal

ID NO: 1AHK

Deposited: 07-Apr-1997 Exp. Method: NMR, Minimized Average Structure

Title	Der F 2, The Major Mite Allergen From Dermatophagoides Farinae, NMR,

Minimized Average Structure

Classification Allergen

Compound Mol_Id: 1; Molecule: Der F 2; Chain: Null; Synonym: Der F II; Engineered: Yes

ID NO: 1AHM

Deposited: 07-Apr-1997 Exp. Method: NMR, 10 Structures

Title Der F 2, The Major Mite Allergen From Dermatophagoides Farinae, NMR, 10 Structures

Classification Allergen

Compound Mol_Id: 1; Molecule: Der F 2; Chain: Null; Synonym: Der F II; Engineered: Yes

ID NO: 1B6F

Deposited: 13-Jan-1999 Exp. Method: NMR, 23 Structures

Title Birch Pollen Allergen Bet V 1

Classification Plant Protein

Compound Mol_Id: 1; Molecule: Major Pollen Allergen Bet V 1-A; Chain: A; Engineered: Yes; Mutation: Yes

ID NO: 1BBG

Deposited: 24-Apr-1998 Exp. Method: NMR, Minimized Average Structure

Title Ragweed Pollen Allergen From Ambrosia Trifida V, NMR, Minimized Average Structure

Classification Allergen

Compound Mol_Id: 1; Molecule: Pollen Allergen 5; Chain: Null

ID NO: 1BJ7

Deposited: 02-Jul-1998 Exp. Method: X-ray Diffraction Resolution: 1.80 Å

Title Bovine Lipocalin Allergen Bos D 2

Classification Allergen

Compound Mol_Id: 1; Molecule: D 2; Chain: Null; Synonym: Dander Major Allergen Bda20, Dermal Allergen Bda20; Engineered: Yes; Biological_Unit: Monomer

ID NO: 1BMW

Deposited: 27-Jul-1998 Exp. Method: NMR, 38 Structures

Title A Fibronectin Type III Fold In Plant Allergens: The Solution Structure Of Phl Pii From Timothy Grass Pollen, NMR, 38 Structures

Classification Allergen

Compound Mol_Id: 1; Molecule: Pollen Allergen Phl P2; Chain: Null; Synonym: Phl P II; Engineered: Yes; Biological_Unit: Monomer

ID NO: 1BTV

Deposited: 30-Jan-1997 Exp. Method: NMR, 20 Structures

Title Structure Of Bet V 1, NMR, 20 Structures

Classification Major Birch Pollen Allergen

Compound Mol_Id: 1; Molecule: Bet V 1; Chain: Null; Engineered: Yes

ID NO: 1BV1

Deposited: 08-Jul-1997 Exp. Method: X-ray Diffraction Resolution: 2.00 Å

Title Birch Pollen Allergen Bet V 1

Classification Allergen

Compound Mol_Id: 1; Molecule: Bet V 1; Chain: Null; Synonym: Major Pollen Allergen Bet V 1-A; Engineered: Yes

ID NO: 1BWH

Deposited: 24-Sep-1998 Exp. Method: X-ray Diffraction Resolution: 1.80 Å

Title The 1.8 A Structure Of Ground Control Grown Tetragonal Hen Egg White Lysozyme

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: Gal D IV, Allergen Gal D 4; Ec: 3.2.1.17

ID NO: 1BWI

Deposited: 24-Sep-1998 Exp. Method: X-ray Diffraction Resolution: 1.80 Å

Title	The 1.8 A Structure Of Microbatch Oil Drop Grown Tetragonal Hen Egg White Lysozyme
Classification	Hydrolase
Compound	Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: Gal D IV, Allergen Gal D 4; Ec: 3.2.1.17

ID NO: 1BWJ

Deposited: 18-Sep-1998 Exp. Method: X-ray Diffraction Resolution: 1.80 Å

Title	The 1.8 A Structure Of Microgravity Grown Tetragonal Hen Egg White Lysozyme
Classification	Hydrolase
Compound	Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: Gal D IV, Allergen Gal D 4; Ec: 3.2.1.17

ID NO: 1CQA

Deposited: 26-Jul-1996 Exp. Method: X-ray Diffraction Resolution: 2.40 Å

Title	Birch Pollen Profilin
Classification	Contractile Protein
Compound	Mol_Id: 1; Molecule: Profilin; Chain: Null; Engineered: Yes

ID NO: 1E09

Deposited: 15-Mar-2000 Exp. Method: NMR, 22 Structures

Title Solution Structure Of The Major Cherry Allergen Pru Av 1

Classification Allergen

Compound Mol_Id: 1; Molecule: Pru Av 1; Chain: A; Engineered: Yes

ID NO: 1EW3

Deposited: 21-Apr-2000 Exp. Method: X-ray Diffraction Resolution: 2.30 Å

Title Crystal Structure Of The Major Horse Allergen Equ C 1

Classification Allergen

Compound Mol_Id: 1; Molecule: Allergen Equ C 1; Chain: A; Engineered: Yes

ID NO: 1F2K

Deposited: 26-May-2000 Exp. Method: X-ray Diffraction Resolution: 2.30 Å

Title Crystal Structure Of Acanthamoeba Castellanii Profilin II, Cubic Crystal Form

Classification Structural Protein

Compound Mol_Id: 1; Molecule: Profilin II; Chain: A, B; Engineered: Yes

ID NO: 1FCQ

Deposited: 19-Jul-2000 Exp. Method: X-ray Diffraction Resolution: 1.60 Å

Title Crystal Structure (Monoclinic) Of Bee Venom Hyaluronidase

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Hyaluronoglucosaminidase; Chain: A; Synonym: Hyaluronidase, Api M II; Ec: 3.2.1.35; Engineered: Yes

ID NO: 1FCU

Deposited: 19-Jul-2000 Exp. Method: X-ray Diffraction Resolution: 2.10 Å

Title Crystal Structure (Trigonal) Of Bee Venom Hyaluronidase

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Hyaluronoglucosaminidase; Chain: A; Synonym: Hyaluronidase, Api M II; Ec: 3.2.1.35; Engineered: Yes

ID NO: 1FCV

Deposited: 19-Jul-2000 Exp. Method: X-ray Diffraction Resolution: 2.65 Å

Title Crystal Structure Of Bee Venom Hyaluronidase In Complex With Hyaluronic Acid Tetramer

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Hyaluronoglucosaminidase; Chain: A; Synonym: Hyaluronidase, Api M II; Ec: 3.2.1.35; Engineered: Yes

ID NO: 1FLQ

Deposited: 15-Aug-2000 Exp. Method: X-ray Diffraction Resolution: 1.80 Å

Title Hen Egg White Lysozyme Mutant With Alanine Substituted For Glycine

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes

ID NO: 1FLU

Deposited: 15-Aug-2000 Exp. Method: X-ray Diffraction Resolution: 1.78 Å

Title Hen Egg White Lysozyme Mutant With Alanine Substituted For Glycine

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes

ID NO: 1FLW

Deposited: 15-Aug-2000 Exp. Method: X-ray Diffraction Resolution: 1.81 Å

Title Hen Egg White Lysozyme Mutant With Alanine Substituted For Glycine

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes

ID NO: 1FLY

Deposited: 15-Aug-2000 Exp. Method: X-ray Diffraction Resolution: 1.83 Å

Title Hen Egg White Lysozyme Mutant With Alanine Substituted For Glycine

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes

ID NO: 1FN5

Deposited: 21-Aug-2000 Exp. Method: X-ray Diffraction Resolution: 1.78 Å

Title Hen Egg White Lysozyme Mutant With Alanine Substituted For Glycine

Classification Hydrolase

Compound Mol_Id: 1; Molecule: Lysozyme; Chain: A; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes

ID NO: 1FSK

Deposited: 11-Sep-2000 Exp. Method: X-ray Diffraction Resolution: 2.90 Å

Title Complex Formation Between A Fab Fragment Of A Monoclonal IgG Antibody and The Major Allergen From Birch Pollen Bet V 1

Classification Immune System

Compound Mol_Id: 1; Molecule: Major Pollen Allergen Bet V 1-A; Chain: A, D, G, J; Synonym: Bet V I-A, Betvi Allergen; Engineered: Yes Mol_Id: 2; Molecule: Immunoglobulin Light Chain; Chain: B, E, H, K; Synonym: Bv16 Fab-Fragment, Mopc21 Coding Sequence; Engineered: Yes Mol_Id: 3; Molecule: Antibody

Heavy Chain Fab; Chain: C, F, I, L; Synonym: Heavy Chain Of The Monoclonal Antibody Mst2; Engineered: Yes

ID NO: 1G5U

Deposited: 02-Nov-2000 Exp. Method: X-ray Diffraction Resolution: 3.10 Å

Title Latex Profilin Hevb8

Classification Allergen

Compound Mol_Id: 1; Molecule: Profilin; Chain: A, B; Engineered: Yes

ID NO: 1H6M

Deposited: 19-Jun-2001 Exp. Method: X-ray Diffraction Resolution: 1.64 Å

Title Covalent Glycosyl-Enzyme Intermediate Of Hen Egg White Lysozyme

Classification Hydrolase (O-Glycosyl)

Compound Mol_Id: 1; Molecule: Lysozyme C; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D 4, Gal D IV; Chain: A; Ec: 3.2.1.17; Engineered: Yes; Mutation: Yes; Other_Details: Covalent 2-Fluorochitobiosyl Enzyme Intermediate

ID NO: 1JTI

Deposited: 21-Aug-2001 Exp. Method: X-ray Diffraction Resolution: 2.30 Å

Title Loop-Inserted Structure Of P1-P1' Cleaved Ovalbumin Mutant R339T

Classification Allergen

Compound Mol_Id: 1; Molecule: Ovalbumin; Chain: A, B; Engineered: Yes; Mutation: Yes

ID NO: 1JTT

Deposited: 22-Aug-2001 Exp. Method: X-ray Diffraction Resolution: 2.10 Å

Title Degenerate Interfaces In Antigen-Antibody Complexes

Classification Immune System, Lysozyme

Compound Mol_Id: 1; Molecule: Vh Single-Domain Antibody; Chain: A; Fragment: Vh Domain Fragment; Engineered: Yes Mol_Id: 2; Molecule: Lysozyme; Chain: L; Fragment: Enzyme; Synonym: 1,4--N-Acetylmuramidase C, Allergen Gal D IV; Ec: 3.2.1.17

ID NO: 1K0K

Deposited: 19-Sep-2001 Exp. Method: X-ray Diffraction Resolution: 2.35 Å

Title Yeast Profilin, Cubic Crystal Form

Classification Contractile Protein

Compound Mol_Id: 1; Molecule: Profilin; Chain: A; Engineered: Yes

ID NO: 1KKC

Deposited: 07-Dec-2001 Exp. Method: X-ray Diffraction Resolution: 2.00 Å

Title Crystal Structure Of Aspergillus Fumigatus Mnsod

Classification Oxidoreductase

Compound Mol_Id: 1; Molecule: Manganese Superoxide Dismutase; Chain: A, B, X, Y; Synonym: Mnsod; Ec: 1.15.1.1; Engineered: Yes

ID NO: 1KUR

Deposited: 22-Jan-2002 Exp. Method: Theoretical Model

Title Theoretical Model Of The Allergen Jun A 3 From Mountain Cedar Pollen

Classification Allergen

Compound Mol_Id: 1; Molecule: Allergen Jun A 3; Chain: A; Synonym: Pathogenesis-Related Protein

ID NO: 1PLM

Deposited: 09-Jan-1998 Exp. Method: Theoretical Model

Title Arabidopsis Profilin 1 Complexed With Poly-L-Proline, Theoretical Model

Classification Complex (Protein/Peptide)

Compound Mol_Id: 1; Molecule: Profilin 1; Chain: A; Engineered: Yes Mol_Id: 2; Molecule: Poly-L-Proline; Chain: B; Engineered: Yes

ID NO: 1PRQ

Deposited: 18-Aug-1997 Exp. Method: X-ray Diffraction Resolution: 2.50 Å

Title Acanthamoeba Castellanii Profilin Ia

Classification Contractile Protein

Compound Mol_Id: 1; Molecule: Profilin Ia; Chain: Null; Engineered: Yes

ID NO: 1QMR

Deposited: 06-Oct-1999 Exp. Method: X-ray Diffraction Resolution: 2.15 Å

Title Birch Pollen Allergen Bet V 1 Mutant N28T, K32Q, E45S, P108G

Classification Allergen

Compound Mol_Id: 1; Molecule: Major Pollen Allergen Bet V 1-A; Chain: A; Synonym: Bet V 1; Engineered: Yes; Mutation: Yes

ID NO: 1QNX

Deposited: 25-Oct-1999 Exp. Method: X-ray Diffraction Resolution: 1.90 Å

Title Ves V 5, An Allergen From Vespula Vulgaris Venom

Classification Allergen

Compound Mol_Id: 1; Molecule: Ves V 5; Chain: A; Synonym: Antigen 5; Engineered: Yes

ID NO: 1WHO

Deposited: 04-Apr-1997 Exp. Method: X-ray Diffraction Resolution: 1.90 Å

Title Allergen Phl P 2

Classification Allergen

Compound Mol_Id: 1; Molecule: Allergen Phl P 2; Chain: Null; Synonym: Phl P II;

Engineered: Yes

ID NO: 1WHP

Deposited: 04-Apr-1997 Exp. Method: X-ray Diffraction Resolution: 3.00 Å

Title Allergen Phl P 2

Classification Allergen

Compound Mol_Id: 1; Molecule: Allergen Phl P 2; Chain: Null; Synonym: Phl P II; Engineered: Yes

ID NO: 2BBG

Deposited: 24-Apr-1998 Exp. Method: NMR, 30 Structures

Title Ragweed Pollen Allergen From Ambrosia Trifida V, NMR, 30 Structures

Classification Allergen

Compound Mol_Id: 1; Molecule: Pollen Allergen 5; Chain: Null

ID NO: 3BBG

Deposited: 24-Apr-1998 Exp. Method: NMR, 2 Structures

Title Multi-Conformer Structure Of Ragweed Pollen Allergen From Ambrosia Trifida V, NMR, 2 Structures

Classification Allergen

Compound Mol_Id: 1; Molecule: Pollen Allergen 5; Chain: Null

ID NO: 3NUL

Deposited: 27-Nov-1996 Exp. Method: X-ray Diffraction Resolution: 1.60 Å

Title Profilin I From Arabidopsis Thaliana

Classification Actin-Binding Protein

Compound Mol_Id: 1; Molecule: Profilin I; Chain: Null; Engineered: Selenomethionyl Protein

We claim:

1. An allergen hybrid protein having reduced allergenicity but retaining immunogenicity, comprising a peptide epitope sequence of an allergen protein and a scaffold protein that is structurally homologous to the allergen protein, wherein the hybrid protein has a native conformation and the peptide epitope sequence is present in a surface accessible region of the hybrid protein corresponding to its position in the allergen protein.

2. The hybrid protein of claim 1 wherein the peptide epitope sequence is in a loop or corner region of the hybrid protein.

3. The hybrid protein of claim 1 wherein the scaffold protein has at least 50 percent sequence identity to the allergen from which the peptide epitope sequence is derived.

4. The hybrid protein of claim 1 wherein the scaffold protein does not have more than 70 percent sequence identity to the allergen protein from which the peptide epitope sequence is derived.

5. The hybrid protein of claim 1 wherein the peptide epitope sequence is about 6 to about 55 amino acids in length.

6. The hybrid protein of claim 5 wherein the peptide epitope sequence is about 6 to about 45 amino acids in length.

7. The hybrid protein of claim 6 wherein the peptide epitope sequence is about 6 to about 35 amino acids in length.

8. The hybrid protein of claim 7 wherein the peptide epitope sequence is about 6 to about 25 amino acids in length.

9. The hybrid protein of claim 8 wherein the peptide epitope sequence is about 6 to about 15 amino acids in length.

10. The hybrid protein of claim 1 further comprising a signal peptide.

11. The hybrid protein of claim 1 further comprising a protease processing site.

12. The hybrid protein of claim 1 which is a hybrid vespid venom allergen protein.

13. The hybrid protein of claim 12, which is a hybrid vespid venom antigen 5 protein.

14. The hybrid protein of claim 13 wherein the peptide epitope sequence is from the genus *Vespula* and the scaffold protein is from the genus *Polistes*.

15. The hybrid protein of claim 14 wherein the peptide epitope sequence is from the species *vulgaris*.

16. The hybrid protein of claim 14 wherein the scaffold protein is from the species *annularis*.

17. The hybrid protein of claim 13 wherein the peptide antigen comprises a sequence selected from the group consisting of

NNYCKIKC (SEQ ID: 1);
NNYCKIKCLKGGVHTACK (SEQ ID: 2);
NNYCKIKCLKGGVHTACKYGSLKP (SEQ ID: 3);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVV (SEQ ID: 4);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQ (SEQ ID: 5);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILK (SEQ ID: 6);
QVGQNVALTGSTAAKYDDPVKLVKMWEDEVKDYNPKKKFSGNDFLKTG (SEQ ID NO: 7);
HYTQMVWANTKEVGCGSIKYIQEKWHKHYLVCNYGPSGNFKNEELYQTK (SEQ ID NO: 8)
LKPNCGNKVVV (SEQ ID NO: 9);

LTGSTAAKYDD (SEQ ID NO: 10);
PKKKFSGND (SEQ ID NO: 11)
IQIKWHK (SEQ ID NO: 12); and
FKNEELYQTK (SEQ ID NO: 13);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILK EHND (SEQ ID NO: 93);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILK EHNDFRQKIAR (SEQ ID NO: 94);
NNYCKIKCLKGGVHTACKYGSLKPNCGNKVVVSYGLTKQEKQDILK EHNDFRQKIARGLETRGNPGPQPPAKNMKN (SEQ ID NO: 95).

18. The hybrid protein of claim 1 wherein the peptide epitope sequence comprises a conservative amino acid change.

19. The hybrid protein of claim 18 wherein the variant peptide is characterized as reducing antibody binding to the peptide epitope sequence by at least 50-percent in an *in vitro* assay, wherein the variant is present in the assay at a concentration less than 10-fold greater than the peptide epitope sequence, and the assay measures binding of the peptide epitope sequence to an antibody directed against a polypeptide comprising the peptide epitope sequence.

20. A nucleic acid encoding the allergen hybrid protein of any one of claims 1-19.

21. A method for preparing a nucleic acid that encodes an allergen hybrid protein; which method comprises introducing a nucleotide sequence encoding a peptide epitope sequence of an allergen protein into a nucleotide sequence encoding a scaffold protein that is structurally homologous to the allergen protein, wherein the nucleotide sequence encoding the peptide epitope sequence is in-frame with the nucleotide sequence encoding the scaffold protein and is in a location such that in the allergen hybrid protein the peptide epitope sequence is present in a surface accessible region of the hybrid protein corresponding to its position in the allergen protein.

22. The method according to claim 21, wherein the nucleotide sequence encoding the scaffold protein is mutated to introduce the nucleotide sequence encoding the peptide epitope sequence.

23. The method according to claim 21, wherein the nucleotide encoding the peptide epitope sequence is introduced by ligating fragments from nucleic acids comprising the nucleotide sequence encoding the peptide epitope sequence and the nucleotide sequence encoding the scaffold protein treated with an endonuclease.

24. A nucleic acid prepared according to the method of claim 21.

25. An expression vector comprising the isolated nucleic acid of claim 20 operationally associated with a promoter.

26. A method for producing an allergen hybrid protein with reduced allergenicity but retaining immunogenicity, which method comprises culturing a cell transformed with the expression vector of claim 25 so that the hybrid allergen is produced by the cell.

27. The method of claim 26, which further comprises recovering the hybrid allergen from the culture, the cell, or both.

28. A method for treating an allergic condition, which method comprises administering a therapeutically effective amount of the hybrid protein of claim 1 or the expression vector of claim 25 to a patient who is allergic to the allergen protein or the scaffold protein, or both.

29. The method of claim 28, wherein the hybrid protein or expression vector is administered orally, pulmonarily, nasally, topically or parenterilly.

30. A pharmaceutical composition comprising the hybrid protein of claim 1 or the expression vector of claim 25 and a pharmaceutically acceptable diluent or carrier.

31. A method of designing a hybrid allergen of reduced allergenicity but retaining immunogenicity, which method comprises

(a) identifying a solvent exposed surface of an allergen;

(b) identifying a protein that is structurally homologous to the allergen; and

(c) modifying sequence of the protein that is structurally homologous to the allergen to incorporate a peptide sequence from the solvent exposed surface of the allergen.

32. The method of claim 31 wherein said solvent exposed surface is identified by a physical means.

33. The method of claim 32 wherein said physical means is x-ray crystallography.

34. The method of claim 31 wherein said solvent exposed surface is identified by comparing the amino acid sequence of the allergen to the amino acid sequence of a structurally homologous protein of known three-dimensional structure.

35. The method of claim 31, wherein the solvent exposed surface comprises a loop or a corner region.

FIG. 1

```
      N N Y C K I K C L K G G V H T A C K Y G 20
1     aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga

      S L K P N C G N K V V V S Y G L T K Q E 40
61    agtcttaaac cgaattgcgg taataaggta gtggtatcct atggtctaac gaaacaagag

      K Q D I L K E H N D F R Q K I A R G L E 60
121   aaacaagaca tcttaaagga gcacaatgac tttagacaaa aaattgcacg aggattggag

      T R G N P G P Q P P A K N M K N L V W N 80
181   actagaggta atcctggacc acagcctcca gcgaagaata tgaaaaattt ggtatggaac

      D E L A Y V A Q V W A N Q C Q Y G H D T 100
241   gacgagttag cttatgtcgc ccaagtgtgg gctaatcaat gtcaatatgg tcacgatact

      C R D V A K Y Q V G Q N V A L T G S T A 120
301   tgcagggatg tagcaaaata tcaggttgga caaaacgtag ccttaacagg tagcacggct

      A K Y D D P V K L V K M W E D E V K D Y 140
361   gctaaatacg atgatccagt taaactagtt aaaatgtggg aagatgaagt gaaagattat

      N P K K K F S G N D F L K T G H Y T Q M 160
421   aatcctaaga aaaagttttc gggaaacgac tttctgaaaa ccggccatta cactcaaatg

      V W A N T K E V G C G S I K Y I Q E K W 180
481   gtttgggcta acaccaagga agttggttgt ggaagtataa aatacattca agagaaatgg

      H K H Y L V C N Y G P S G N F K N E E L 200
541   cacaaacatt accttgtatg taattatgga cccagcggaa actttaagaa tgaggaactt

      Y Q T K *                                                        204
601   tatcaaacaa agtaa
```

FIG. 3

```
Ves NNYCKIKCLK GGVHTACKYG .SLKP..NCG NKVVVSYGLT KQEKQDILKE
Pol VDYCKIKC.P SGIHTVCQYG ESTKPSKNCA GKVIKSVGPT EEEKKLIVSE

Ves HNDFRQKIAR GLETRGNPGP QPPAKNMKNL VWNDELAYVA QVWANQCQY.
Pol HNRFRQKVAQ GLETRGNPGP QPAASDMNDL VWNDELAHIA QVWASQCQFL

Ves GHDTCRDVAK YQVGQNVALT GSTAAKYDDP VKLVKMWEDE VKDYNPKKKF
Pol VHDKCRNTAK YPVGQNIAYA G..GSNLPDV VSLIKLWENE VKDFNYNTGI

Ves SGNDFLKTGH YTQMVWANTK EVGCGSIKYI QEKWHKHYLV CNYGPSGNFK
Pol TKQNFAKIGH YTQMVWGKTK EIGCGSLKYM ENNMQNHYLI CNYGPAGNYL

Ves NEELYQTK 204
Pol GQLPYTKK 205
```

FIG. 2

```
     V  D  Y  C   K  I  K   C  P  S   G  I  H  T   V  C  Q   Y  G  E    20
1    gttgattatt gtaaaataaa gtgtccaagt ggtatccata cagtctgcca atatggagaa

     S  T  K  P   S  K  N   C  A  G   K  V  I  K   S  V  G   P  T  E    40
61   tcgacaaaac caagcaagaa ttgtgccggt aaagtaatca aatcggttgg tccaacggaa

     E  E  K  K   L  I  V   S  E  H   N  R  F  R   Q  K  V   A  Q  G    60
121  gaggagaaaa aattaatcgt aagcgagcat aatcggttta gacaaaaagt tgcacagggg

     L  E  T  R   G  N  P   G  P  Q   P  A  A  S   D  M  N   D  L  V    80
181  ttggaaacaa gaggtaatcc tggaccacaa cctgctgcct cggacatgaa tgatttggta

     W  N  D  E   L  A  H   I  A  Q   V  W  A  S   Q  C  Q   F  L  V    100
241  tggaacgatg aattagcaca tatcgcgcaa gtatgggcca gccaatgcca atttctcgta

     H  D  K  C   R  N  T   A  K  Y   P  V  G  Q   N  I  A   Y  A  G    120
301  cacgacaaat gcaggaatac cgcaaaatat ccagttggac aaaatatagc gtatgcaggt

     G  S  N  L   P  D  V   V  S  L   I  K  L  W   E  N  E   V  K  D    140
361  ggttctaact taccagatgt agtcagtcta atcaaacttt gggaaaacga agtgaaagat

     F  N  Y  N   T  G  I   T  K  Q   N  F  A  K   I  G  H   Y  T  Q    160
421  tttaattaca atacaggaat aacaaaacaa aactttgcta aaattggcca ttacactcaa

     M  V  W  G   K  T  K   E  I  G   C  G  S  L   K  Y  M   E  N  N    180
481  atggtttggg gtaaaactaa agaaattggt tgtggatctc taaaatatat ggaaaataat

     M  Q  N  H   Y  L  I   C  N  Y   G  P  A  G   N  Y  L   G  Q  L    200
541  atgcaaaatc attacctcat atgtaattat ggaccagctg gaaattactt gggtcaacta

     P  Y  T  K   K  *                                                  205
601  ccttatacaa aaaaataa
```

FIG. 4

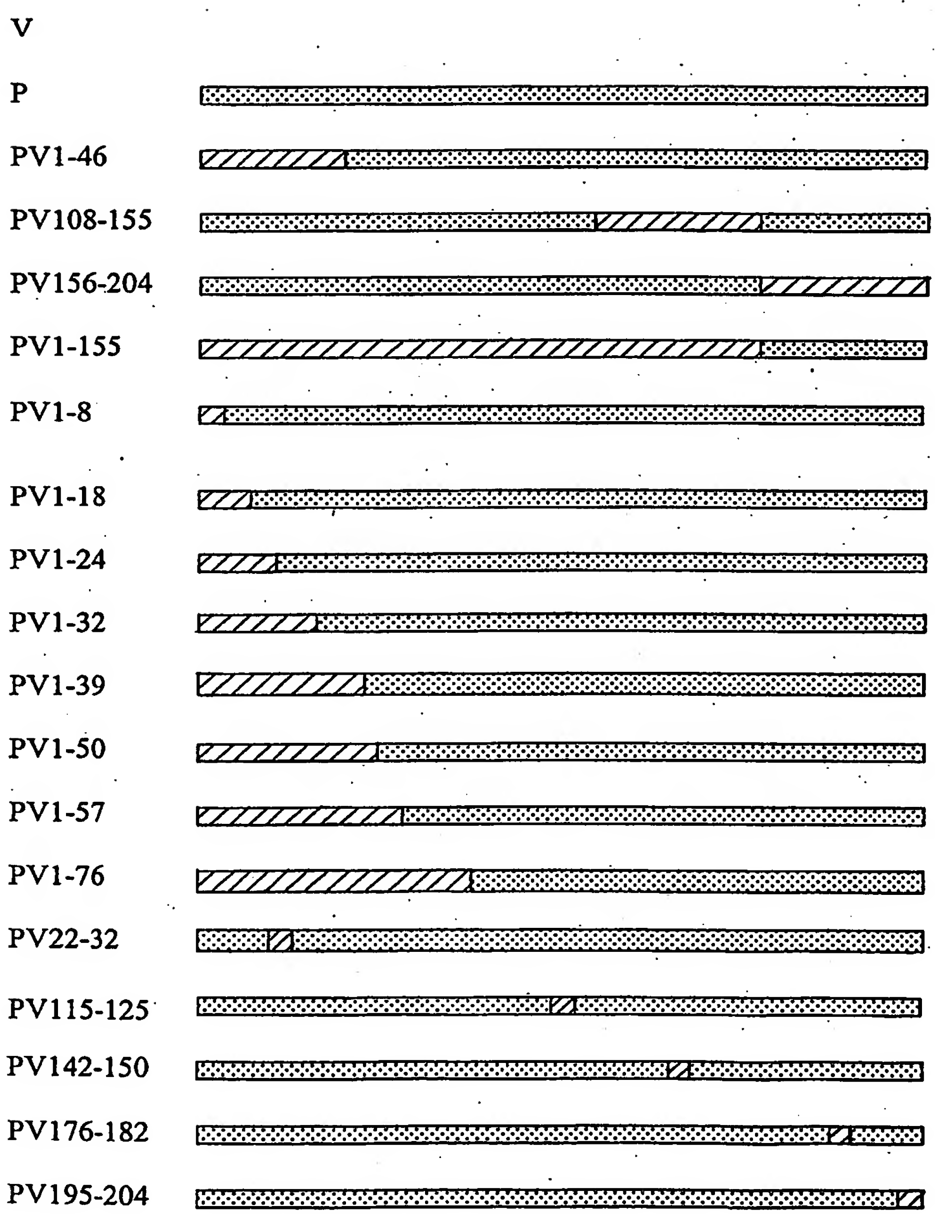
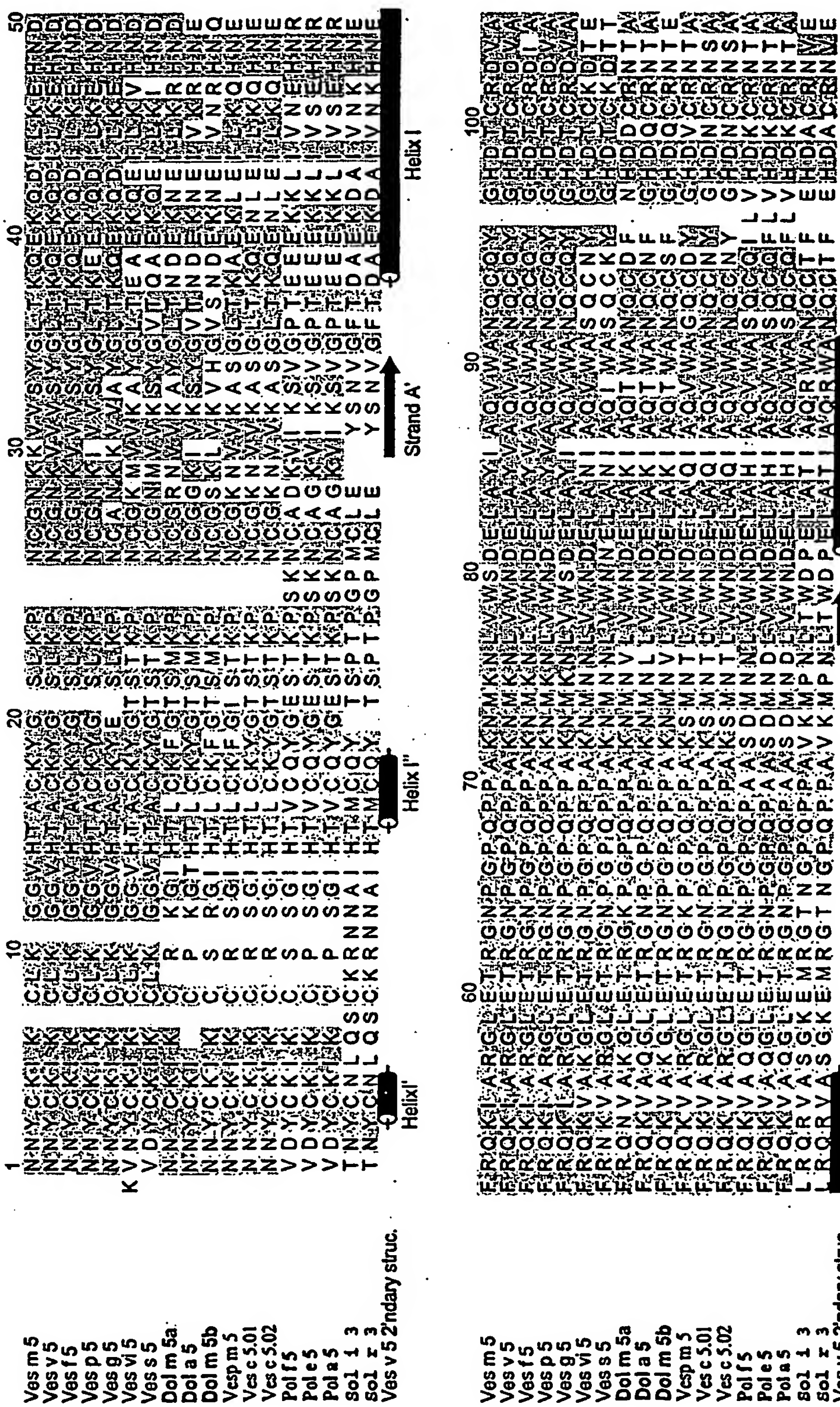
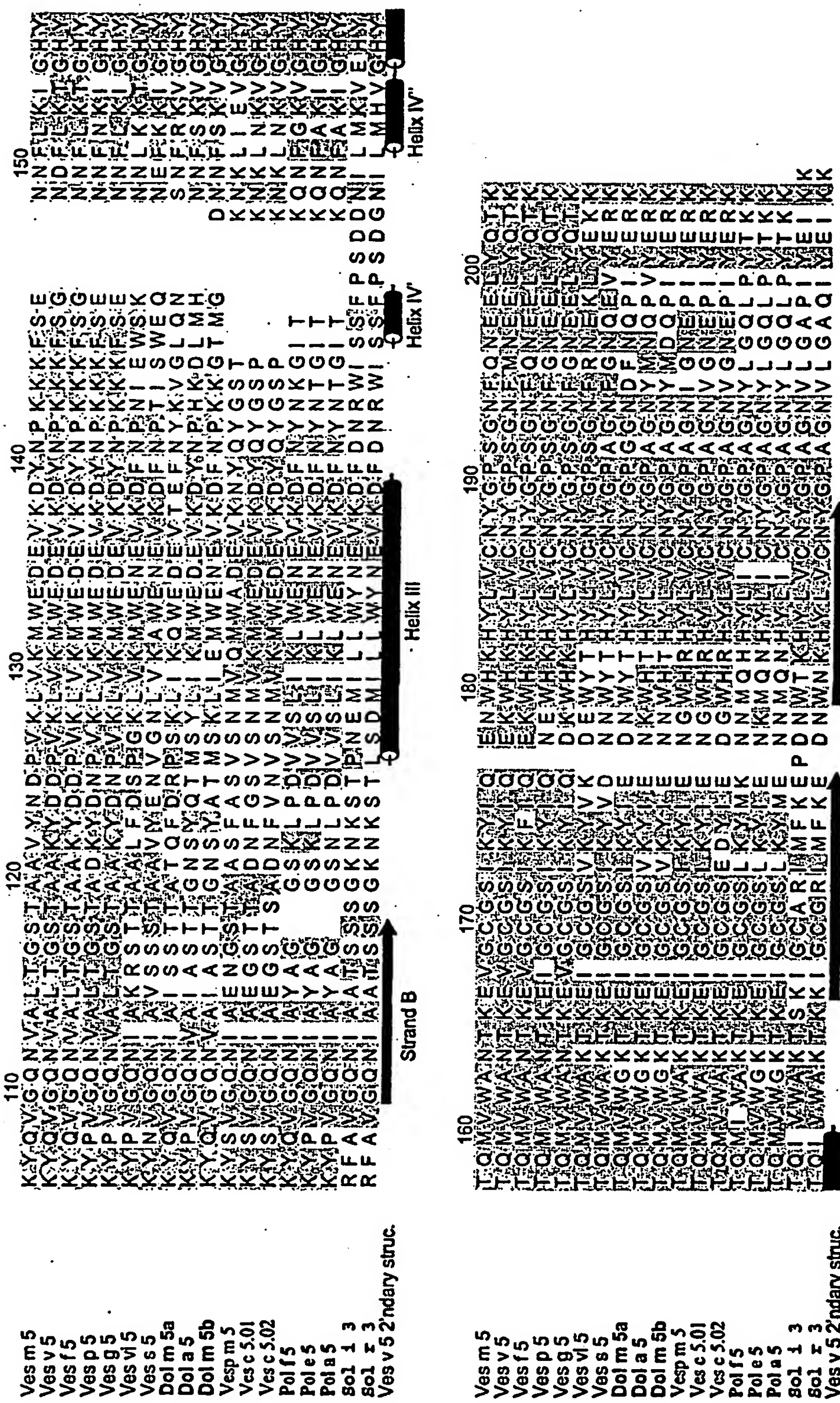

V
P
PV1-46
PV108-155
PV156-204
PV1-155
PV1-8
PV1-18
PV1-24
PV1-32
PV1-39
PV1-50
PV1-57
PV1-76
PV22-32
PV115-125
PV142-150
PV176-182
PV195-204

FIG. 5A



BEST AVAILABLE COPY

FIG. 5B



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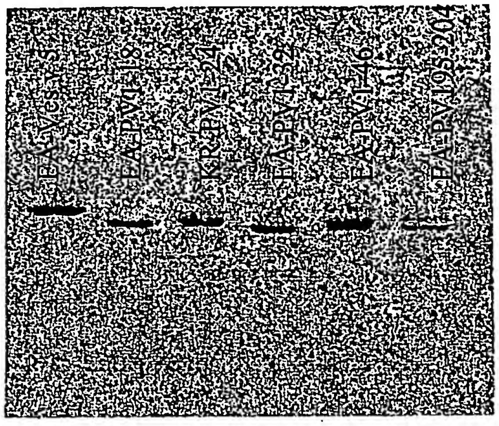


FIG. 6A

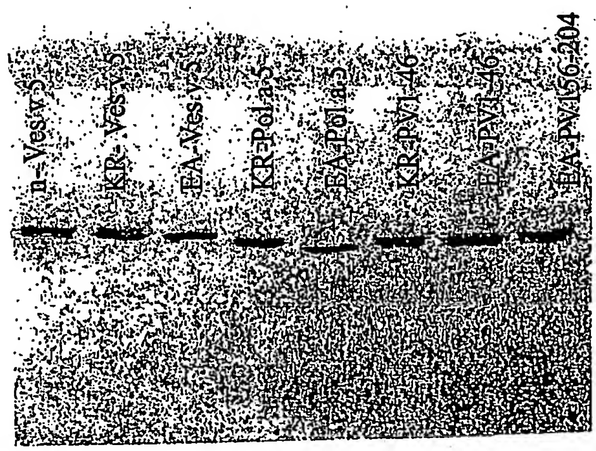
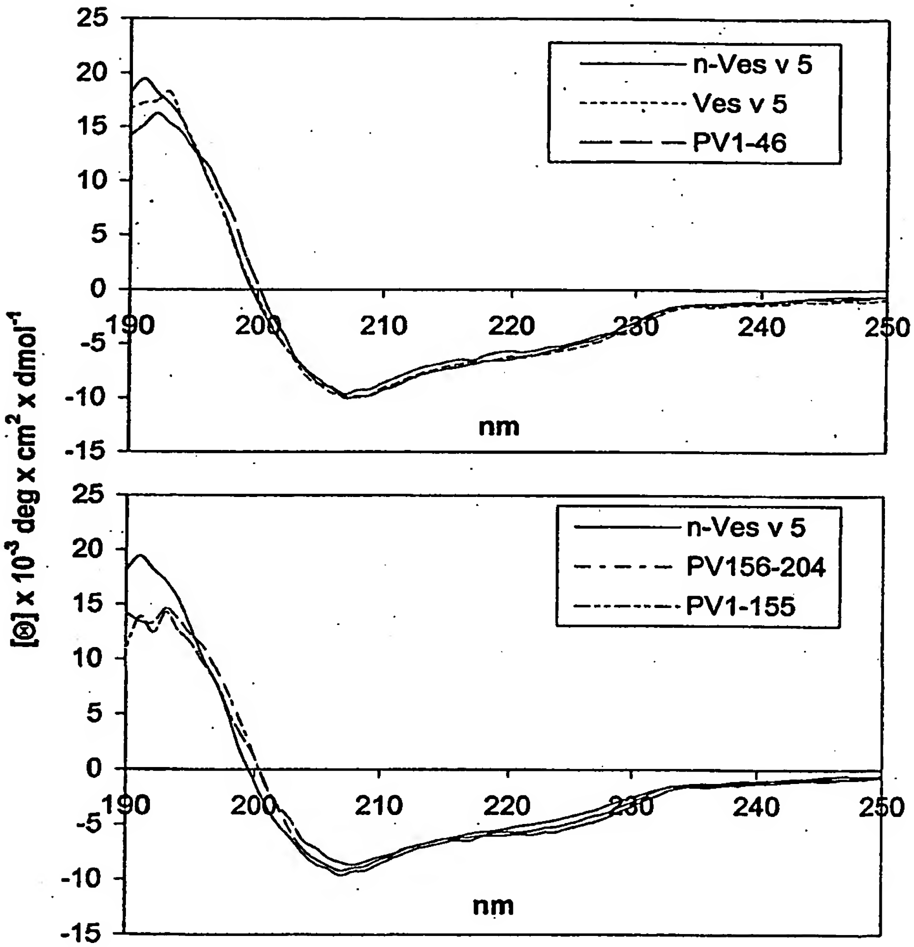


FIG. 6B

FIG. 7



25
20
15
10
5
0
-5
-10
-15
190
200
210
220
230
240
250
nm
n-Ves v 5
Ves v 5
PV1-46
[Θ] x 10^-3 deg x cm^2 x dmol^-1
n-Ves v 5
PV156-204
PV1-155

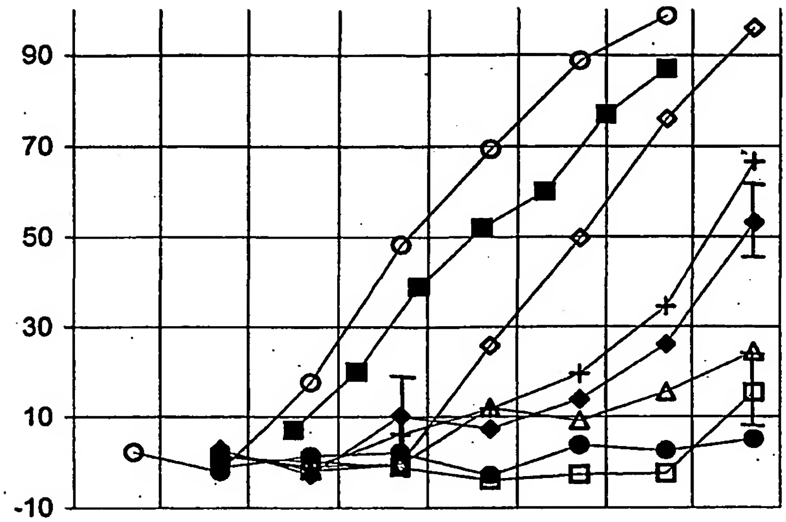


FIG. 8A

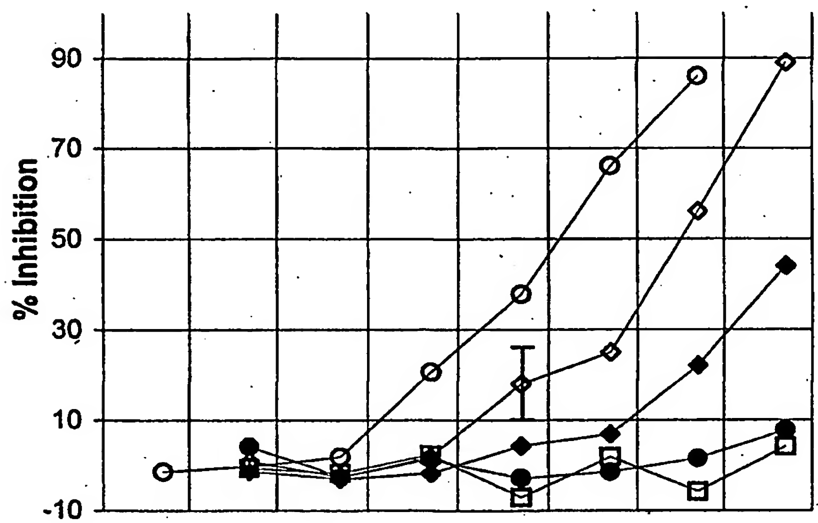


FIG. 8B

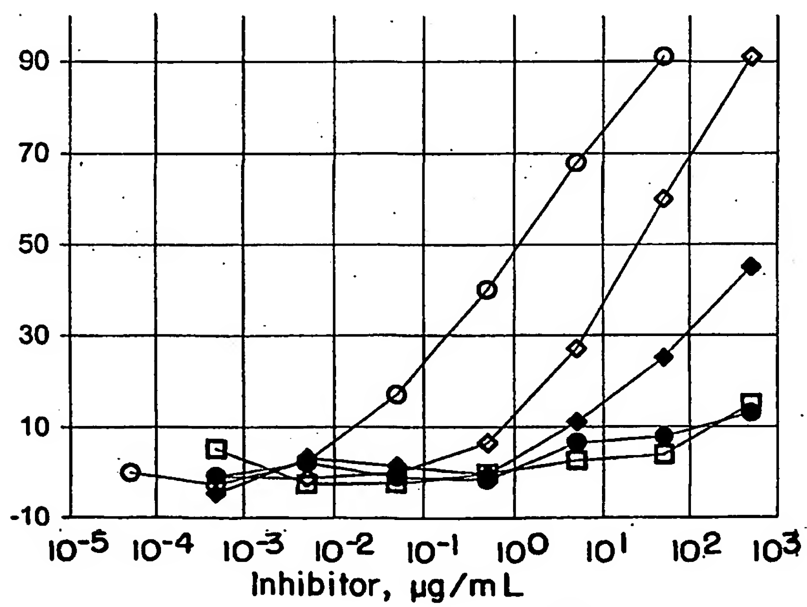


FIG. 8C

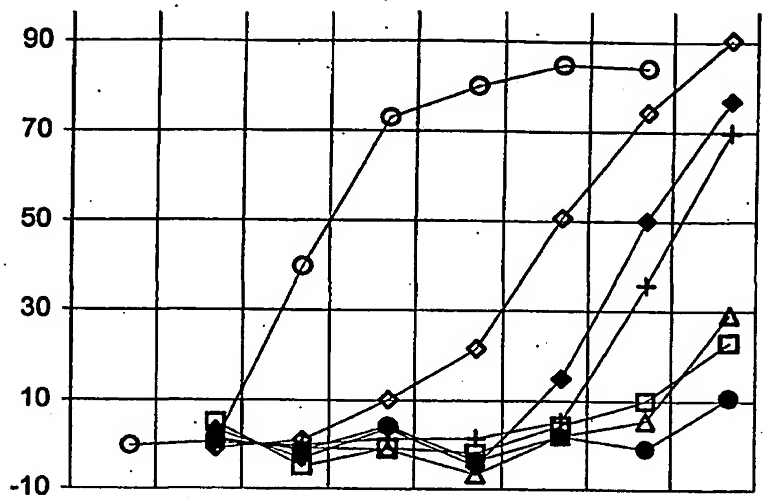


FIG. 9A

EA- or KR-Ves v 5
EA-PV1-46
EA-PV1-18
EA-PV1-155
KR-PV1-24
EA-PV156-204

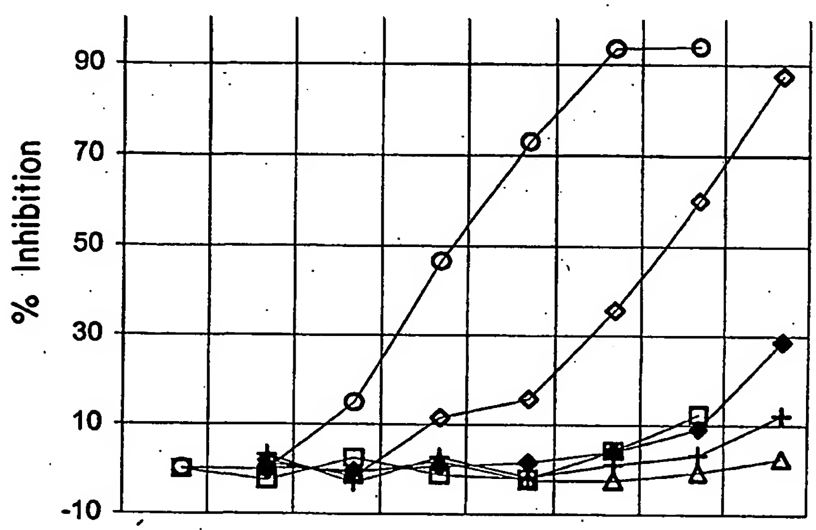


FIG. 9B

EA- or KR-Ves v 5
EA-PV1-46
EA-PV1-18
EA-PV1-155
KR-PV1-24
EA-PV156-204

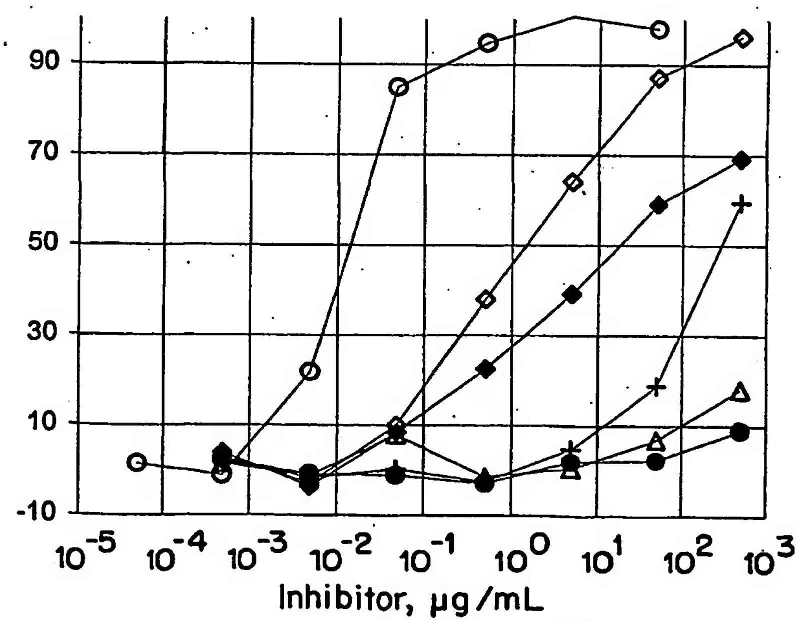


FIG. 9C

EA- or KR-Ves v 5
EA-PV1-46
EA-PV1-18
EA-PV1-155
KR-PV1-24
EA-PV156-204

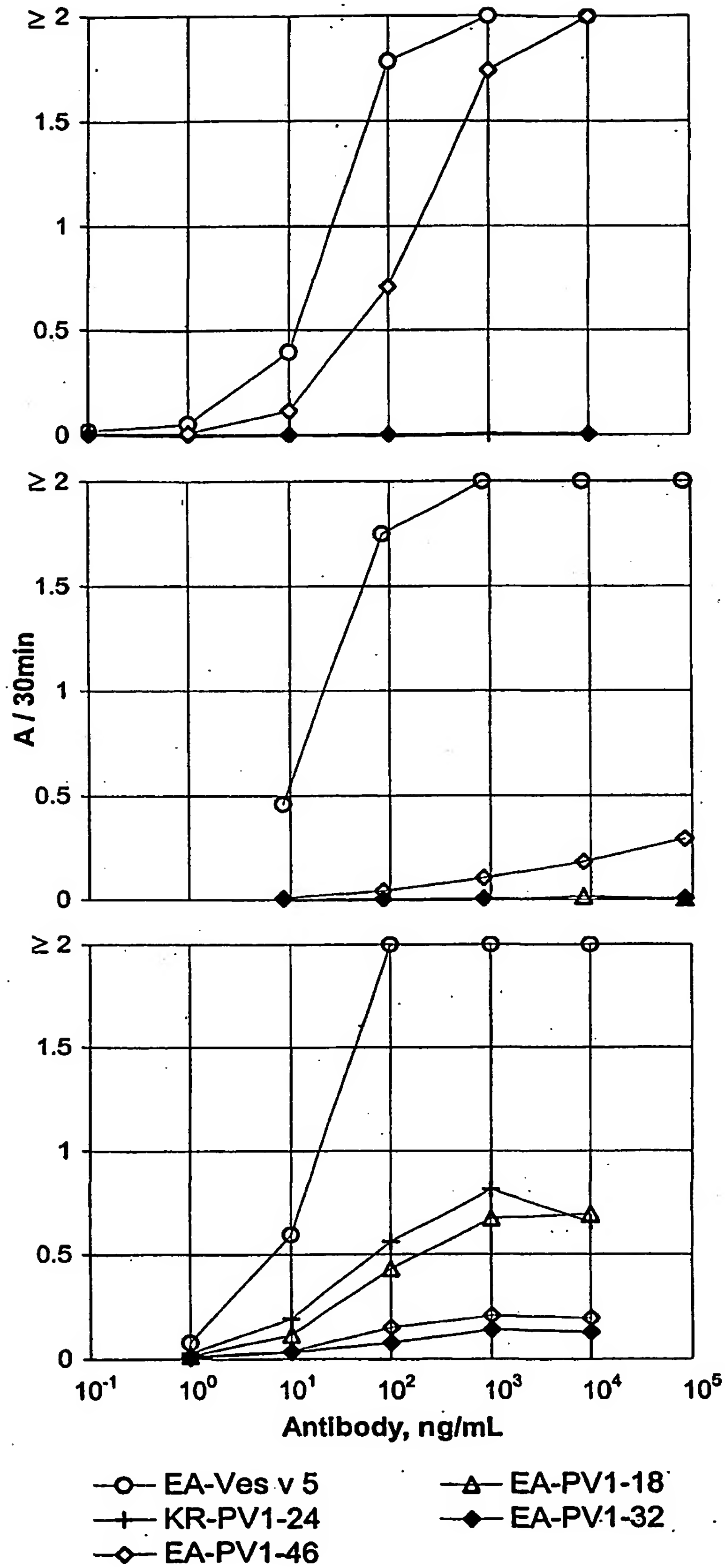


FIG. 10A

FIG. 10B

FIG. 10C

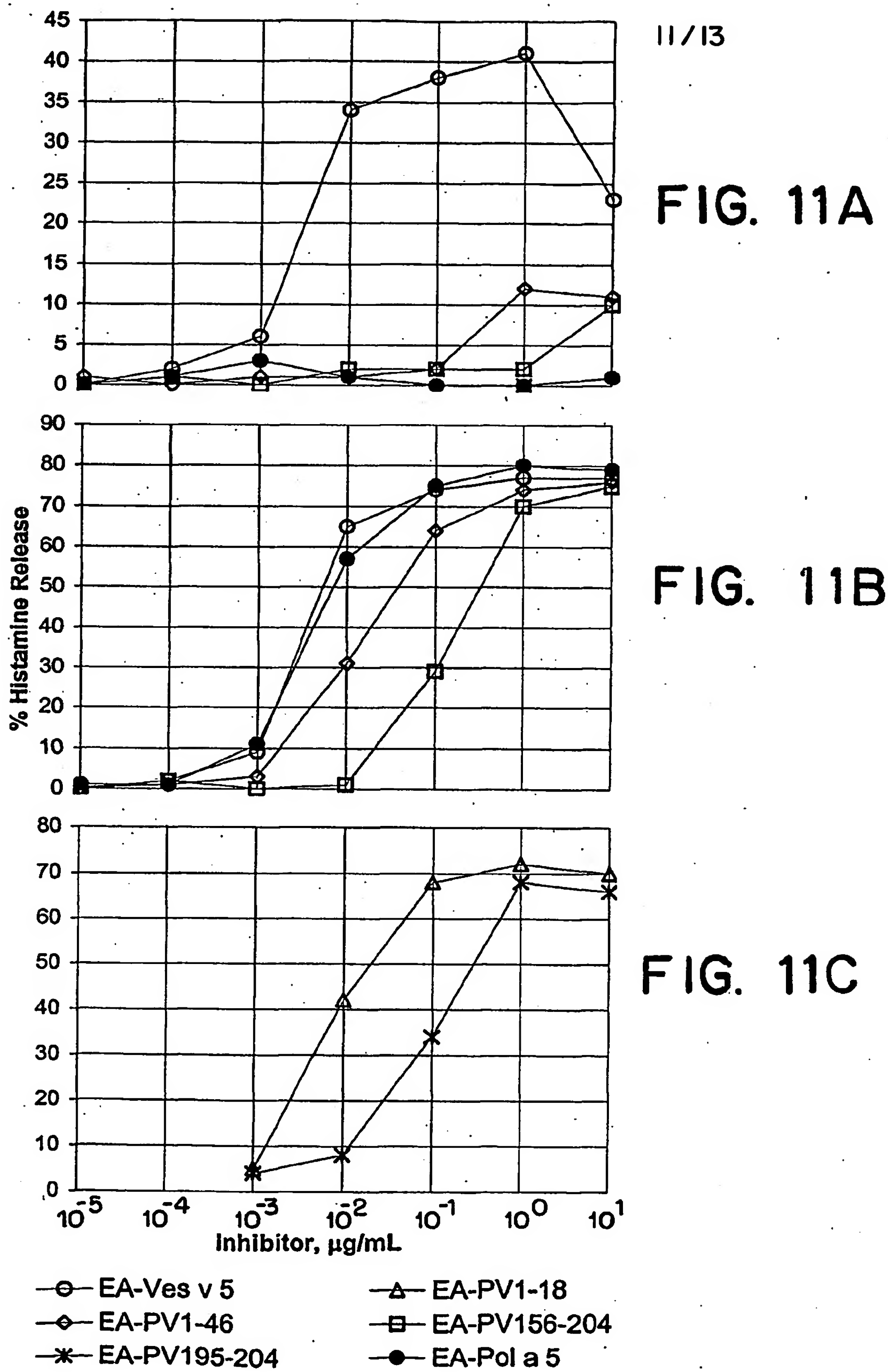


FIG. 11A
FIG. 11B
FIG. 11C
% Histamine Release
45
40
35
30
25
20
15
10
5
0
90
80
70
60
50
40
30
20
10
0
80
70
60
50
40
30
20
10
0
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
Inhibitor, µg/mL
EA-Ves v 5
EA-PV1-18
EA-PV1-46
EA-PV156-204
EA-PV195-204
EA-Pol a 5

FIG. 12A

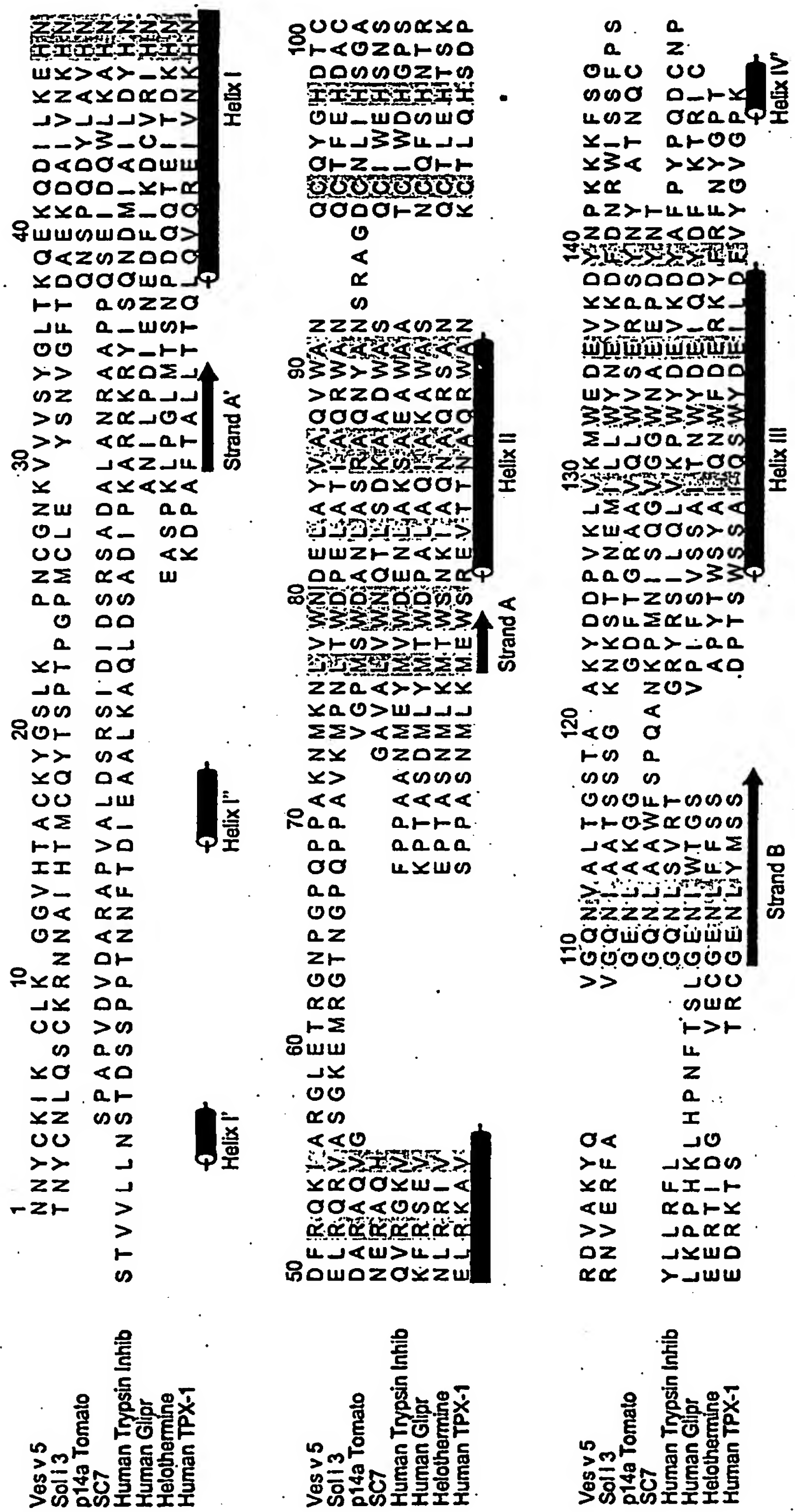

Ves v 5
Sol I 3
p14a Tomato
SC7
Human Trypsin Inhib
Human Glipr
Helothermine
Human TPX-1
Helix I'
Helix I''
Strand A'
Helix I
Strand A
Helix II
Strand B
Helix III
Helix IV'

FIG. 12B

```
                              200
Ves v 5               PSGNFMNEELYQTK
Sol i 3               PAGNVLGAPIYEIKK
p14a Tomato           PVGNWIGQRPY
SC7                   RPGNVSPRDKYYPINVQP
Human Trypsin Inhib   PKGNWIGE  APYKVGVPCSSCPPSYG GSCTDNLCFPGVTSNYLYWFK
Human Glipr           PGGNYPT   WPYKRGATCSACPNN   DKCLDNLCVNRQRDQVKRYYSVVYPGWP
Helothermine          PGGNIRSRKYTPYSIGPPCGDCPDACDNGLCT NPCKQNDVYNNCPDLKKQVGCGH
Human TPX-1           PAGNNMNRKNTPYQQGTPCAGCPDDCDKGLCT NSCQYQDLLSNCDSLKNTAGCEH

Ves v 5
Sol i 3
p14a Tomato
SC7
Human Trypsin Inhib
Human Glipr           IYPRNRYTSLFLIVNSVILILSVIITILVQLKYPNLVLLD
Helothermine          PIMKD CMATCKCLTEIK
Human TPX-1           ELLKEKCKATCLCENKIY
```

SEQUENCE LISTING

<110> The Rockefeller University
ALK Abelló

<120> RECOMBINANT HYBRID ALLERGEN CONSTRUCTS WITH REDUCED ALLERGENICITY THAT RETAIN IMMUNOGENICITY OF THE NATURAL ALLERGEN

<130> 2313/2H587WO0

<150> US 60/272,818
<151> 2001-03-02

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<170> PatentIn version 3.1

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<212> PRT
<213> Vespula vulgaris

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1               5
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<400> 2

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1               5                   10                  15

Cys Lys
```

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<211> 24
<212> PRT
<213> Vespula vulgaris

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1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro
20

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<213> Vespula vulgaris

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1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

<210> 5
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<213> Vespula vulgaris

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Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Gln
35

<210> 6
<211> 46
<212> PRT
<213> Vespula vulgaris

<400> 6

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala

1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys
35 40 45

<210> 7
<211> 48
<212> PRT
<213> Vespula vulgaris

<400> 7

Gln Val Gly Gln Asn Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr
1 5 10 15

Asp Asp Pro Val Lys Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp
20 25 30

Tyr Asn Pro Lys Lys Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly
35 40 45

<210> 8
<211> 49
<212> PRT
<213> Vespula vulgaris

<400> 8

His Tyr Thr Gln Met Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly
1 5 10 15

Ser Ile Lys Tyr Ile Gln Glu Lys Trp His Lys His Tyr Leu Val Cys
20 25 30

Asn Tyr Gly Pro Ser Gly Asn Phe Lys Asn Glu Glu Leu Tyr Gln Thr
35 40 45

Lys

<210> 9
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<400> 9

Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
1   5   10

<210> 10
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1   5   10

<210> 11
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Pro Lys Lys Lys Phe Ser Gly Asn Asp
1   5

<210> 12
<211> 7
<212> PRT
<213> Vespula vulgaris

<400> 12

Ile Gln Ile Lys Trp His Lys
1   5

<210> 13
<211> 10
<212> PRT

<213> Vespula vulgaris

<400> 13

Phe Lys Asn Glu Glu Leu Tyr Gln Thr Lys
1 5 10

<210> 14
<211> 615
<212> DNA
<213> Vespula vulgaris

<400> 14
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga 60

agtcttaaac cgaattgcgg taataaggta gtggtatcct atggtctaac gaaacaagag 120

aaacaagaca tcttaaagga gcacaatgac tttagacaaa aaattgcacg aggattggag 180

actagaggta atcctggacc acagcctcca gcgaagaata tgaaaaattt ggtatggaac 240

gacgagttag cttatgtcgc ccaagtgtgg gctaatcaat gtcaatatgg tcacgatact 300

tgcagggatg tagcaaaata tcaggttgga caaaacgtag ccttaacagg tagcacggct 360

gctaaatacg atgatccagt taaactagtt aaaatgtggg aagatgaagt gaaagattat 420

aatcctaaga aaaagttttc gggaaacgac tttctgaaaa ccggccatta cactcaaatg 480

gtttgggcta acaccaagga agttggttgt ggaagtataa aatacattca agagaaatgg 540

cacaaacatt accttgtatg taattatgga cccagcggaa actttaagaa tgaggaactt 600

tatcaaacaa agtaa 615

<210> 15
<211> 618
<212> DNA
<213> Polistes annularis

<400> 15
gttgattatt gtaaaataaa gtgtccaagt ggtatccata cagtctgcca atatggagaa 60

tcgacaaaac caagcaagaa ttgtgccggt aaagtaatca aatcggttgg tccaacggaa 120

gaggagaaaa aattaatcgt aagcgagcat aatcggttta gacaaaaagt tgcacagggg 180

ttggaaacaa gaggtaatcc tggaccacaa cctgctgcct cggacatgaa tgatttggta 240

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tggaacgatg aattagcaca tatcgcgcaa gtatgggcca gccaatgcca atttctcgta     300

cacgacaaat gcaggaatac cgcaaaatat ccagttggac aaaatatagc gtatgcaggt     360

ggttctaact taccagatgt agtcagtcta atcaaacttt gggaaaacga agtgaaagat     420

tttaattaca atacaggaat aacaaaacaa aactttgcta aaattggcca ttacactcaa     480

atggtttggg gtaaaactaa agaaattggt tgtggatctc taaaatatat ggaaaataat     540

atgcaaaatc attacctcat atgtaattat ggaccagctg gaaattactt gggtcaacta     600

ccttatacaa aaaaataa                                                   618

<210>  16
<211>  204
<212>  PRT
<213>  Vespula vulgaris

<400>  16

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1               5                   10                  15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
            20                  25                  30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
        35                  40                  45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Asn
65                  70                  75                  80

Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
                85                  90                  95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Gln Val Gly Gln Asn
            100                 105                 110
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Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr Asp Asp Pro Val Lys
        115                 120                 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
    130                 135                 140

Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly His Tyr Thr Gln Met
145                 150                 155                 160

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Tyr Ile
                165                 170                 175

Gln Glu Lys Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
            180                 185                 190

Gly Asn Phe Lys Asn Glu Glu Leu Tyr Gln Thr Lys
        195                 200

<210>  17
<211>  205
<212>  PRT
<213>  Polistes annularis

<400>  17

Val Asp Tyr Cys Lys Ile Lys Cys Pro Ser Gly Ile His Thr Val Cys
1               5                   10                  15

Gln Tyr Gly Glu Ser Thr Lys Pro Ser Lys Asn Cys Ala Gly Lys Val
            20                  25                  30

Ile Lys Ser Val Gly Pro Thr Glu Glu Glu Lys Lys Leu Ile Val Ser
        35                  40                  45

Glu His Asn Arg Phe Arg Gln Lys Val Ala Gln Gly Leu Glu Thr Arg
    50                  55                  60

Gly Asn Pro Gly Pro Gln Pro Ala Ala Ser Asp Met Asn Asp Leu Val
65                  70                  75                  80
```

Trp Asn Asp Glu Leu Ala His Ile Ala Gln Val Trp Ala Ser Gln Cys
85 90 95

Gln Phe Leu Val His Asp Lys Cys Arg Asn Thr Ala Lys Tyr Pro Val
100 105 110

Gly Gln Asn Ile Ala Tyr Ala Gly Gly Ser Asn Leu Pro Asp Val Val
115 120 125

Ser Leu Ile Lys Leu Trp Glu Asn Glu Val Lys Asp Phe Asn Tyr Asn
130 135 140

Thr Gly Ile Thr Lys Gln Asn Phe Ala Lys Ile Gly His Tyr Thr Gln
145 150 155 160

Met Val Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Leu Lys Tyr
165 170 175

Met Glu Asn Asn Met Gln Asn His Tyr Leu Ile Cys Asn Tyr Gly Pro
180 185 190

Ala Gly Asn Tyr Leu Gly Gln Leu Pro Tyr Thr Lys Lys
195 200 205

<210> 18
<211> 24
<212> DNA
<213> Vespula vulgaris

<400> 18
aacaattatt gtaaaataaa atgt 24

<210> 19
<211> 54
<212> DNA
<213> Vespula vulgaris

<400> 19
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaa 54

<210> 20
<211> 72
<212> DNA
<213> Vespula vulgaris

<400> 20
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga 60

agtcttaaac cg 72

<210> 21
<211> 96
<212> DNA
<213> Vespula vulgaris

<400> 21
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga 60

agtcttaaac cgaattgcgg taataaggta gtggta 96

<210> 22
<211> 117
<212> DNA
<213> Vespula vulgaris

<400> 22
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga 60

agtcttaaac cgaattgcgg taataaggta gtggtatcct atggtctaac gaaacaa 117

<210> 23
<211> 138
<212> DNA
<213> Vespula vulgaris

<400> 23
aacaattatt gtaaaataaa atgtttgaaa ggaggtgtcc atactgcctg caaatatgga 60

agtcttaaac cgaattgcgg taataaggta gtggtatcct atggtctaac gaaacaagag 120

aaacaagaca tcttaaag 138

<210> 24
<211> 144

<212> DNA
<213> Vespula vulgaris

<400> 24
caggttggac aaaacgtagc cttaacaggt agcacggctg ctaaatacga tgatccagtt 60

aaactagtta aaatgtggga agatgaagtg aaagattata atcctaagaa aaagttttcg 120

ggaaacgact ttctgaaaac cggc 144

<210> 25
<211> 147
<212> DNA
<213> Vespula vulgaris

<400> 25
cattacactc aaatggtttg ggctaacacc aaggaagttg gttgtggaag tataaaatac 60

attcaagaga aatggcacaa acattacctt gtatgtaatt atggacccag cggaaacttt 120

aagaatgagg aactttatca aacaaag 147

<210> 26
<211> 33
<212> DNA
<213> Vespula vulgaris

<400> 26
cttaaaccga attgcggtaa taaggtagtg gta 33

<210> 27
<211> 33
<212> DNA
<213> Vespula vulgaris

<400> 27
ttaacaggta gcacggctgc taaatacgat gat 33

<210> 28
<211> 27
<212> DNA
<213> Vespula vulgaris

<400> 28
cctaagaaaa agttttcggg aaacgac 27

<210> 29
<211> 21
<212> DNA
<213> Vespula vulgaris

<400> 29
attcaagaga aatggcacaa a 21

<210> 30
<211> 30
<212> DNA
<213> Vespula vulgaris

<400> 30
tttaagaatg aggaacttta tcaaacaaag 30

<210> 31
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Ves v 5' EA sense PCR primer 1

<400> 31
cgtgaattca acaattattg taaaataaaa 30

<210> 32
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Ves v 5' KR sense PCR primer 2

<400> 32
cgtctcgaga aaagaaacaa ttattgtaaa ataaaa 36

<210> 33
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Ves v 3' downstream antisense PCR primer 3

<400> 33
cgttctagat tactttgttt gataaagttc 30

<210> 34
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a 5' EA sense PCR primer 4

<400> 34
cgtgaattcg ttgattattg taaaataaaa 30

<210> 35
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a 5' KR sense PCR primer 5

<400> 35
cgtctcgaga aaagagttga ttattgtaaa ataaaa 36

<210> 36
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a 3' downstream antisense primer 6

<400> 36
cgttctagat tatttttttg tataaggtag 30

<210> 37
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a aa 49-50 EH-mutagenic sense PCR primer 7

<400> 37

gtaagcgagc acaatcggtt t 21

<210> 38
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a aa 49-50 EH-mutagenic antisense PCR primer 8

<400> 38
aaaccgattg tgctcgctta c 21

<210> 39
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Ves v ApoI conversion PCR primer 9

<400> 39
gtagcaaaat ttcaggttgg a 21

<210> 40
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Ves v ApoI conversion PCR primer 10

<400> 40
tccaacctga aattttgcta c 21

<210> 41
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a ApoI conversion PCR primer 11

<400> 41
accgcaaaat ttccagttgg a 21

<210> 42
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Pol a ApoI conversion PCR primer 12

<400> 42
tccaactgga aattttgcgg t 21

<210> 43
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-18 sense PCR primer 13

<400> 43
cgtgaattca acaattattg taaaataaaa tgtttgaaag gaggtgtcca tactgcctgc 60

aaatatggag aa 72

<210> 44
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> PV195-204 antisense PCR primer 14

<400> 44
cgttctagat tactttgttt gataaagttc ctcattctta aaatttccag ctgg 54

<210> 45
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PV18-24 and PV1-24 antisense PCR primer 15

<400> 45
ggcacaattc ttgctcggtt taagacttcc ata 33

<210> 46
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PV18-24 and PV 1-24 sense PCR primer 16

<400> 46
tatggaagtc ttaaaccgag caagaattgt gcc 33

<210> 47
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PV22-32 and PV1-32 sense PCR primer 17

<400> 47
cttaaaccga attgcggtaa taaggtagtg gtatcggttg gtcca 45

<210> 48
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PV22-32 and PV1-32 antisense PCR primer 18

<400> 48
tggaccaacc gataccacta ccttattacc gcaattcggt ttaag 45

<210> 49
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-39 PCR primer 19

<400> 49
tatggtctaa cgaaacaaga gaaaaaatta atcgta 36

<210> 50

<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-39 PCR primer 20

<400> 50
tacgattaat tttttctctt gtttcgttag accata 36

<210> 51
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PV115-125 sense PCR primer 21

<400> 51
ttaacaggta gcacggctgc taaatacgat gatgtagtca gtcta 45

<210> 52
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PV115-125 antisense PCR primer 22

<400> 52
atcatcgtat ttagcagccg tgctacctgt taacgctata ttttg 45

<210> 53
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> PV142-150 sense PCR primer 23

<400> 53
cctaagaaaa agttttcggg aaacgacttt gctaaaattg gc 42

<210> 54
<211> 42
<212> DNA

<213> Artificial Sequence

<220>
<223> PV142-150 antisense PCR primer 24

<400> 54
gtcgtttccc gaaaactttt tcttaggatt aaaatctttc ac 42

<210> 55
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PV176-182 sense PCR primer 25

<400> 55
attcaagaga aatggcacaa acattacctc ata 33

<210> 56
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PV176-182 antisense PCR primer 26

<400> 56
tttgtgccat ttctcttgaa tatattttag aga 33

<210> 57
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-50 antisense PCR primer 27

<400> 57
gagcacaatg actttagaca aaaa 24

<210> 58
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-57 antisense PCR primer 28

<400> 58
tttttgtcta aagtcattgt gctc 24

<210> 59
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PV1-76 antisense PCR primer 29

<400> 59
aaaattgcac gagggttgga aaca 24

<210> 60
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 60
tgtttccaac cctcgtgcaa tttt 24

<210> 61
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 61
aatatgaaaa atttggtatg gaac 24

<210> 62
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 62
gttccatacc aaatttttca tatt 24

<210> 63
<211> 204
<212> PRT
<213> Vespula maculifrons

<400> 63

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Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1               5                   10                  15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Lys Val Val
            20                  25                  30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
        35                  40                  45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Ser
65                  70                  75                  80

Asp Glu Leu Ala Tyr Ile Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
                85                  90                  95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Gln Val Gly Gln Asn
            100                 105                 110

Val Ala Leu Thr Gly Ser Thr Ala Ala Val Tyr Asn Asp Pro Val Lys
        115                 120                 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
    130                 135                 140

Lys Phe Ser Glu Asn Asn Phe Leu Lys Ile Gly His Tyr Thr Gln Met
145                 150                 155                 160
```

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Tyr Ile
165 170 175

Gln Glu Asn Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
180 185 190

Gly Asn Phe Gln Asn Glu Glu Leu Tyr Gln Thr Lys
195 200

<210> 64
<211> 204
<212> PRT
<213> Vespula vulgaris

<400> 64

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
35 40 45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
50 55 60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Asn
65 70 75 80

Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
85 90 95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Gln Val Gly Gln Asn
100 105 110

Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr Asp Asp Pro Val Lys

115 120 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
130 135 140

Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly His Tyr Thr Gln Met
145 150 155 160

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Tyr Ile
165 170 175

Gln Glu Lys Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
180 185 190

Gly Asn Phe Met Asn Glu Glu Leu Tyr Gln Thr Lys
195 200

<210> 65
<211> 204
<212> PRT
<213> Vespula flavopilosa

<400> 65

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
35 40 45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
50 55 60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Asn
65 70 75 80

Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
85 90 95

Gly His Asp Thr Cys Arg Asp Ile Ala Lys Tyr Gln Val Gly Gln Asn
100 105 110

Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr Asp Asp Pro Val Lys
115 120 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
130 135 140

Lys Phe Ser Gly Asn Asn Phe Leu Lys Thr Gly His Tyr Thr Gln Met
145 150 155 160

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Phe Ile
165 170 175

Gln Glu Lys Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
180 185 190

Gly Asn Phe Gln Asn Glu Glu Leu Tyr Gln Thr Lys
195 200

<210> 66
<211> 204
<212> PRT
<213> Vespula pensylvanica

<400> 66

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Ile Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Glu Glu Lys Gln Asp Ile Leu Lys Glu His
35 40 45

```
Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Asn
65                  70                  75                  80

Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
                85                  90                  95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Pro Val Gly Gln Asn
            100                 105                 110

Val Ala Leu Thr Gly Ser Thr Ala Asp Lys Tyr Asp Asn Pro Val Lys
        115                 120                 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
    130                 135                 140

Lys Phe Ser Glu Asn Asn Phe Asn Lys Ile Gly His Tyr Thr Gln Met
145                 150                 155                 160

Val Trp Ala Asn Thr Lys Glu Ile Gly Cys Gly Ser Ile Lys Tyr Ile
                165                 170                 175

Gln Asn Glu Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
            180                 185                 190

Gly Asn Phe Gly Asn Glu Glu Leu Tyr Gln Thr Lys
        195                 200

<210>  67
<211>  204
<212>  PRT
<213>  Vespula germanica

<400>  67

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1               5                   10                  15
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Cys Lys Tyr Glu Ser Leu Lys Pro Asn Cys Ala Asn Lys Lys Val Val
20 25 30

Ala Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
35 40 45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
50 55 60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Ser
65 70 75 80

Asp Glu Leu Ala Tyr Ile Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
85 90 95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Pro Val Gly Gln Asn
100 105 110

Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr Asp Asn Pro Val Lys
115 120 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
130 135 140

Lys Phe Ser Glu Asn Asn Phe Leu Lys Ile Gly His Tyr Thr Gln Met
145 150 155 160

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Tyr Ile
165 170 175

Gln Asp Lys Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
180 185 190

Gly Asn Phe Gly Asn Glu Glu Leu Tyr Gln Thr Lys
195 200

<210> 68
<211> 206
<212> PRT
<213> Vespula vidua

<400> 68

Lys Val Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr
1 5 10 15

Ala Cys Lys Tyr Gly Thr Ser Thr Lys Pro Asn Cys Gly Lys Met Val
20 25 30

Val Lys Ala Tyr Gly Leu Thr Glu Ala Glu Lys Gln Glu Ile Leu Lys
35 40 45

Val His Asn Asp Phe Arg Gln Lys Val Ala Lys Gly Leu Glu Thr Arg
50 55 60

Gly Asn Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Asn Asn Leu Val
65 70 75 80

Trp Asn Asp Glu Leu Ala Asn Ile Ala Gln Val Trp Ala Ser Gln Cys
85 90 95

Asn Tyr Gly His Asp Thr Cys Lys Asp Thr Glu Lys Tyr Pro Val Gly
100 105 110

Gln Asn Ile Ala Lys Arg Ser Thr Thr Ala Ala Leu Phe Asp Ser Pro
115 120 125

Gly Lys Leu Val Lys Met Trp Glu Asn Glu Val Lys Asp Phe Asn Pro
130 135 140

Asn Ile Glu Trp Ser Lys Asn Asn Leu Lys Lys Thr Gly His Tyr Thr
145 150 155 160

Gln Met Val Trp Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Val Lys
165 170 175

```
Tyr Val Lys Asp Glu Trp Tyr Thr His Tyr Leu Val Cys Asn Tyr Gly
            180                 185                 190

Pro Ser Gly Asn Phe Arg Asn Glu Lys Leu Tyr Glu Lys Lys
        195                 200                 205


<210>  69
<211>  205
<212>  PRT
<213>  Vespula squamosa

<400>  69

Val Asp Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1               5                   10                  15

Cys Lys Tyr Gly Thr Ser Thr Lys Pro Asn Cys Gly Asn Met Val Val
            20                  25                  30

Lys Ser Tyr Gly Val Thr Gln Ala Glu Lys Gln Glu Ile Leu Lys Ile
        35                  40                  45

His Asn Asp Phe Arg Asn Lys Val Ala Arg Gly Leu Glu Thr Arg Gly
    50                  55                  60

Asn Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Asn Asn Leu Val Trp
65                  70                  75                  80

Asn Asn Glu Leu Ala Asn Ile Ala Gln Ile Trp Ala Ser Gln Cys Lys
                85                  90                  95

Tyr Gly His Asp Thr Cys Lys Asp Thr Thr Lys Tyr Asn Val Gly Gln
            100                 105                 110

Asn Ile Ala Val Ser Ser Ser Thr Ala Ala Val Tyr Glu Asn Val Gly
        115                 120                 125

Asn Leu Val Lys Ala Trp Glu Asn Glu Val Lys Asp Phe Asn Pro Thr
    130                 135                 140
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```
Ile Ser Trp Glu Gln Asn Glu Phe Lys Lys Ile Gly His Tyr Thr Gln
145                 150                 155                 160

Met Val Trp Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Ile Lys Tyr
                165                 170                 175

Val Asp Asn Asn Trp Tyr Thr His Tyr Leu Val Cys Asn Tyr Gly Pro
            180                 185                 190

Ala Gly Asn Phe Gly Asn Gln Glu Val Tyr Glu Arg Lys
        195                 200                 205


<210>  70
<211>  204
<212>  PRT
<213>  Dolichovespula maculata

<400>  70

Asn Asn Tyr Cys Lys Ile Lys Cys Arg Lys Gly Ile His Thr Leu Cys
1               5                   10                  15

Lys Phe Gly Thr Ser Met Lys Pro Asn Cys Gly Arg Asn Val Val Lys
            20                  25                  30

Ala Tyr Gly Leu Thr Asn Asp Glu Lys Asn Glu Ile Leu Lys Arg His
        35                  40                  45

Asn Asp Phe Arg Gln Asn Val Ala Lys Gly Leu Glu Thr Arg Gly Lys
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Asn Val Leu Val Trp Asn
65                  70                  75                  80

Asp Glu Leu Ala Lys Ile Ala Gln Thr Trp Ala Asn Gln Cys Asp Phe
                85                  90                  95

Asn His Asp Asp Cys Arg Asn Thr Ala Lys Tyr Gln Val Gly Gln Asn
```

100 105 110

Ile Ala Ile Ser Ser Thr Thr Ala Thr Gln Phe Asp Arg Pro Ser Lys
115 120 125

Leu Ile Lys Gln Trp Glu Asp Glu Val Thr Glu Phe Asn Tyr Lys Val
130 135 140

Gly Leu Gln Asn Ser Asn Phe Arg Lys Val Gly His Tyr Thr Gln Met
145 150 155 160

Val Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Ile Lys Tyr Ile
165 170 175

Glu Asp Asn Trp Tyr Thr His Tyr Leu Val Cys Asn Tyr Gly Pro Gly
180 185 190

Gly Asn Asp Phe Asn Gln Pro Ile Tyr Glu Arg Lys
195 200

<210> 71
<211> 203
<212> PRT
<213> Dolichovespula arenaria

<400> 71

Asn Asn Tyr Cys Lys Ile Cys Pro Lys Gly Thr His Thr Leu Cys Lys
1 5 10 15

Tyr Gly Thr Ser Met Lys Pro Asn Cys Gly Gly Lys Ile Val Lys Ser
20 25 30

Tyr Gly Val Thr Asn Asp Glu Lys Asn Glu Ile Val Lys Arg His Asn
35 40 45

Glu Phe Arg Gln Lys Val Ala Gln Gly Leu Glu Thr Arg Gly Asn Pro
50 55 60

Gly Pro Gln Pro Pro Ala Lys Asn Met Asn Leu Leu Val Trp Asn Asp
65 70 75 80

Glu Leu Ala Lys Ile Ala Gln Thr Trp Ala Asn Gln Cys Asn Phe Gly
85 90 95

His Asp Gln Cys Arg Asn Thr Ala Lys Tyr Pro Val Gly Gln Asn Val
100 105 110

Ala Ile Ala Ser Thr Thr Gly Asn Ser Tyr Gln Thr Met Ser Tyr Leu
115 120 125

Ile Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro His Lys Asp
130 135 140

Leu Met His Asn Asn Phe Ser Lys Val Gly His Tyr Thr Gln Met Val
145 150 155 160

Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Val Lys Tyr Ile Glu
165 170 175

Asn Lys Trp His Thr His Tyr Leu Val Cys Asn Tyr Gly Pro Ala Gly
180 185 190

Asn Tyr Met Asn Gln Pro Val Tyr Glu Arg Lys
195 200

<210> 72
<211> 205
<212> PRT
<213> Dolichovespula maculata

<400> 72

Asn Asn Tyr Cys Lys Ile Lys Cys Ser Arg Gly Ile His Thr Leu Cys
1 5 10 15

Lys Phe Gly Thr Ser Met Lys Pro Asn Cys Gly Ser Lys Leu Val Lys
20 25 30

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Val His Gly Val Ser Asn Asp Glu Lys Asn Glu Ile Val Asn Arg His
        35                  40                  45

Asn Gln Phe Arg Gln Lys Val Ala Lys Gly Leu Glu Thr Arg Gly Asn
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Asn Val Leu Val Trp Asn
65                  70                  75                  80

Asp Glu Leu Ala Lys Ile Ala Gln Thr Trp Ala Asn Gln Cys Ser Phe
                85                  90                  95

Gly His Asp Gln Cys Arg Asn Thr Glu Lys Tyr Gln Val Gly Gln Asn
            100                 105                 110

Val Ala Ile Ala Ser Thr Thr Gly Asn Ser Tyr Ala Thr Met Ser Lys
        115                 120                 125

Leu Ile Glu Met Trp Glu Asn Glu Val Lys Asp Phe Asn Pro Lys Lys
    130                 135                 140

Gly Thr Met Gly Asp Asn Asn Phe Ser Lys Val Gly His Tyr Thr Gln
145                 150                 155                 160

Met Val Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Val Lys Tyr
                165                 170                 175

Ile Glu Asn Asn Trp His Thr His Tyr Leu Val Cys Asn Tyr Gly Pro
            180                 185                 190

Ala Gly Asn Tyr Met Asp Gln Pro Ile Tyr Glu Arg Lys
        195                 200                 205
```

<210> 73
<211> 202
<212> PRT
<213> Vespa mandarinia

<400> 73

```
Asn Asn Tyr Cys Lys Ile Lys Cys Arg Ser Gly Ile His Thr Leu Cys
1               5                   10                  15

Lys Phe Gly Ile Ser Thr Lys Pro Asn Cys Gly Lys Asn Val Val Lys
            20                  25                  30

Ala Ser Gly Leu Thr Lys Ala Glu Lys Leu Glu Ile Leu Lys Gln His
        35                  40                  45

Asn Glu Phe Arg Gln Lys Val Ala Arg Gly Leu Glu Thr Arg Gly Lys
    50                  55                  60

Pro Gly Pro Gln Pro Pro Ala Lys Ser Met Asn Thr Leu Val Trp Asn
65                  70                  75                  80

Asp Glu Leu Ala Gln Ile Ala Gln Val Trp Ala Gly Gln Cys Asp Tyr
                85                  90                  95

Gly His Asp Val Cys Arg Asn Thr Ala Lys Tyr Ser Val Gly Gln Asn
            100                 105                 110

Ile Ala Glu Asn Gly Ser Thr Ala Ala Ser Phe Ala Ser Val Ser Asn
        115                 120                 125

Met Val Gln Met Trp Ala Asp Glu Val Lys Asn Tyr Gln Tyr Gly Ser
    130                 135                 140

Thr Lys Asn Lys Leu Ile Glu Val Gly His Tyr Thr Gln Met Val Trp
145                 150                 155                 160

Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Ile Lys Tyr Ile Glu Asn
                165                 170                 175

Gly Trp His Arg His Tyr Leu Val Cys Asn Tyr Gly Pro Ala Gly Asn
            180                 185                 190
```

Ile Gly Asn Glu Pro Ile Tyr Glu Arg Lys
195 200

<210> 74
<211> 202
<212> PRT
<213> Vespa crabro

<400> 74

Asn Asn Tyr Cys Lys Ile Lys Cys Arg Ser Gly Ile His Thr Leu Cys
1 5 10 15

Lys Tyr Gly Thr Ser Thr Lys Pro Asn Cys Gly Lys Asn Val Val Lys
20 25 30

Ala Ser Gly Leu Thr Lys Gln Glu Asn Leu Glu Ile Leu Lys Gln His
35 40 45

Asn Glu Phe Arg Gln Lys Val Ala Arg Gly Leu Glu Thr Arg Gly Asn
50 55 60

Pro Gly Pro Gln Pro Pro Ala Lys Ser Met Asn Thr Leu Val Trp Asn
65 70 75 80

Asp Glu Leu Ala Gln Ile Ala Gln Val Trp Ala Asn Gln Cys Asn Tyr
85 90 95

Gly His Asp Asn Cys Arg Asn Ser Ala Lys Tyr Ser Val Gly Gln Asn
100 105 110

Ile Ala Glu Gly Ser Thr Thr Ala Asp Asn Phe Gly Ser Val Ser Asn
115 120 125

Met Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Gln Tyr Gly Ser
130 135 140

Pro Lys Asn Lys Leu Asn Lys Val Gly His Tyr Thr Gln Met Val Trp
145 150 155 160

Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Ile Lys Tyr Ile Glu Asn
165 170 175

Gly Trp His Arg His Tyr Leu Val Cys Asn Tyr Gly Pro Ala Gly Asn
180 185 190

Val Gly Asn Glu Pro Ile Tyr Glu Arg Lys
195 200

<210> 75
<211> 202
<212> PRT
<213> Vespa crabro

<400> 75

Asn Asn Tyr Cys Lys Ile Lys Cys Arg Ser Gly Ile His Thr Leu Cys
1 5 10 15

Lys Tyr Gly Thr Ser Thr Lys Pro Asn Cys Gly Lys Asn Val Val Lys
20 25 30

Ala Ser Gly Leu Thr Lys Gln Glu Asn Leu Glu Ile Leu Lys Gln His
35 40 45

Asn Glu Phe Arg Gln Lys Val Ala Arg Gly Leu Glu Thr Arg Gly Asn
50 55 60

Pro Gly Pro Gln Pro Pro Ala Lys Ser Met Asn Thr Leu Val Trp Asn
65 70 75 80

Asp Glu Leu Ala Gln Ile Ala Gln Val Trp Ala Asn Gln Cys Asn Tyr
85 90 95

Gly His Asp Asn Cys Arg Asn Ser Ala Lys Tyr Ser Val Gly Gln Asn
100 105 110

Ile Ala Glu Gly Ser Thr Ser Ala Asp Asn Phe Val Asn Val Ser Asn
115 120 125

Met Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Gln Tyr Gly Ser
    130   135   140

Pro Lys Asn Lys Leu Asn Lys Val Gly His Tyr Thr Gln Met Val Trp
145   150   155   160

Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Glu Asp Tyr Ile Glu Asp
                165   170   175

Gly Trp His Arg His Tyr Leu Val Cys Asn Tyr Gly Pro Ala Gly Asn
            180   185   190

Val Gly Asn Glu Pro Ile Tyr Glu Arg Lys
        195   200

<210> 76
<211> 205
<212> PRT
<213> Polistes fuscatus

<400> 76

Val Asp Tyr Cys Lys Ile Lys Cys Ser Ser Gly Ile His Thr Val Cys
1   5   10   15

Gln Tyr Gly Glu Ser Thr Lys Pro Ser Lys Asn Cys Ala Asp Lys Val
            20   25   30

Ile Lys Ser Val Gly Pro Thr Glu Glu Glu Lys Lys Leu Ile Val Asn
        35   40   45

Glu His Asn Arg Phe Arg Gln Lys Val Ala Gln Gly Leu Glu Thr Arg
    50   55   60

Gly Asn Pro Gly Pro Gln Pro Ala Ala Ser Asp Met Asn Asn Leu Val
65   70   75   80

Trp Asn Asp Glu Leu Ala His Ile Ala Gln Val Trp Ala Ser Gln Cys

```
                85                  90                  95

Gln Ile Leu Val His Asp Lys Cys Arg Asn Thr Ala Lys Tyr Gln Val
            100                 105                 110

Gly Gln Asn Ile Ala Tyr Ala Gly Gly Ser Lys Leu Pro Asp Val Val
        115                 120                 125

Ser Leu Ile Lys Leu Trp Glu Asn Glu Val Lys Asp Phe Asn Tyr Asn
    130                 135                 140

Lys Gly Ile Thr Lys Gln Asn Phe Gly Lys Val Gly His Tyr Thr Gln
145                 150                 155                 160

Met Ile Trp Ala Lys Thr Lys Glu Ile Gly Cys Gly Ser Leu Lys Tyr
                165                 170                 175

Met Lys Asn Asn Met Gln His His Tyr Leu Ile Cys Asn Tyr Gly Pro
            180                 185                 190

Ala Gly Asn Tyr Leu Gly Gln Leu Pro Tyr Thr Lys Lys
        195                 200                 205

<210>  77
<211>  205
<212>  PRT
<213>  Polistes exclamans

<400>  77

Val Asp Tyr Cys Lys Ile Lys Cys Pro Ser Gly Ile His Thr Val Cys
1               5                   10                  15

Gln Tyr Gly Glu Ser Thr Lys Pro Ser Lys Asn Cys Ala Gly Lys Val
            20                  25                  30

Ile Lys Ser Val Gly Pro Thr Glu Glu Glu Lys Lys Leu Ile Val Ser
        35                  40                  45
```

Glu His Asn Arg Phe Arg Gln Lys Val Ala Gln Gly Leu Glu Thr Arg
    50   55   60

Gly Asn Pro Gly Pro Gln Pro Ala Ala Ser Asp Met Asn Asp Leu Val
65   70   75   80

Trp Asn Asp Glu Leu Ala His Ile Ala Gln Val Trp Ala Ser Gln Cys
                85   90   95

Gln Phe Leu Val His Asp Lys Cys Arg Asn Thr Ala Lys Tyr Pro Val
            100   105   110

Gly Gln Asn Ile Ala Tyr Ala Gly Gly Ser Lys Leu Pro Asp Val Val
        115   120   125

Ser Leu Ile Lys Leu Trp Glu Asn Glu Val Lys Asp Phe Asn Tyr Asn
    130   135   140

Thr Gly Ile Thr Lys Gln Asn Phe Ala Lys Ile Gly His Tyr Thr Gln
145   150   155   160

Met Val Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Leu Lys Tyr
                165   170   175

Ile Glu Asn Lys Met Gln Asn His Tyr Leu Ile Cys Asn Tyr Gly Pro
            180   185   190

Ala Gly Asn Tyr Leu Gly Gln Leu Pro Tyr Thr Lys Lys
        195   200   205

<210> 78
<211> 205
<212> PRT
<213> Polistes annularis

<400> 78

Val Asp Tyr Cys Lys Ile Lys Cys Pro Ser Gly Ile His Thr Val Cys
1   5   10   15

```
Gln Tyr Gly Glu Ser Thr Lys Pro Ser Lys Asn Cys Ala Gly Lys Val
            20                  25                  30

Ile Lys Ser Val Gly Pro Thr Glu Glu Glu Lys Lys Leu Ile Val Ser
        35                  40                  45

Glu His Asn Arg Phe Arg Gln Lys Val Ala Gln Gly Leu Glu Thr Arg
    50                  55                  60

Gly Asn Pro Gly Pro Gln Pro Ala Ala Ser Asp Met Asn Asp Leu Val
65                  70                  75                  80

Trp Asn Asp Glu Leu Ala His Ile Ala Gln Val Trp Ala Ser Gln Cys
                85                  90                  95

Gln Phe Leu Val His Asp Lys Cys Arg Asn Thr Ala Lys Tyr Pro Val
            100                 105                 110

Gly Gln Asn Ile Ala Tyr Ala Gly Gly Ser Asn Leu Pro Asp Val Val
        115                 120                 125

Ser Leu Ile Lys Leu Trp Glu Asn Glu Val Lys Asp Phe Asn Tyr Asn
    130                 135                 140

Thr Gly Ile Thr Lys Gln Asn Phe Ala Lys Ile Gly His Tyr Thr Gln
145                 150                 155                 160

Met Val Trp Gly Lys Thr Lys Glu Ile Gly Cys Gly Ser Leu Lys Tyr
                165                 170                 175

Met Glu Asn Asn Met Gln Asn His Tyr Leu Ile Cys Asn Tyr Gly Pro
            180                 185                 190

Ala Gly Asn Tyr Leu Gly Gln Leu Pro Tyr Thr Lys Lys
        195                 200                 205
```

<210> 79

<211> 212
<212> PRT
<213> Solenopsis invicta

<400> 79

Thr Asn Tyr Cys Asn Leu Gln Ser Cys Lys Arg Asn Asn Ala Ile His
1 5 10 15

Thr Met Cys Gln Tyr Thr Ser Pro Thr Pro Gly Pro Met Cys Leu Glu
20 25 30

Tyr Ser Asn Val Gly Phe Thr Asp Ala Glu Lys Asp Ala Ile Val Asn
35 40 45

Lys His Asn Glu Leu Arg Gln Arg Val Ala Ser Gly Lys Glu Met Arg
50 55 60

Gly Thr Asn Gly Pro Gln Pro Pro Ala Val Lys Met Pro Asn Leu Thr
65 70 75 80

Trp Asp Pro Glu Leu Ala Thr Ile Ala Gln Arg Trp Ala Asn Gln Cys
85 90 95

Thr Phe Glu His Asp Ala Cys Arg Asn Val Glu Arg Phe Ala Val Gly
100 105 110

Gln Asn Ile Ala Ala Thr Ser Ser Ser Gly Lys Asn Lys Ser Thr Pro
115 120 125

Asn Glu Met Ile Leu Leu Trp Tyr Asn Glu Val Lys Asp Phe Asp Asn
130 135 140

Arg Trp Ile Ser Ser Phe Pro Ser Asp Asp Asn Ile Leu Met Lys Val
145 150 155 160

Glu His Tyr Thr Gln Ile Val Trp Ala Lys Thr Ser Lys Ile Gly Cys
165 170 175

```
Ala Arg Ile Met Phe Lys Glu Pro Asp Asn Trp Thr Lys His Tyr Leu
            180                 185                 190

Val Cys Asn Tyr Gly Pro Ala Gly Asn Val Leu Gly Ala Pro Ile Tyr
        195                 200                 205

Glu Ile Lys Lys
    210


<210>  80
<211>  211
<212>  PRT
<213>  Solenopsis richteri

<400>  80

Thr Asn Tyr Cys Asn Leu Gln Ser Cys Lys Arg Asn Asn Ala Ile His
1               5                   10                  15

Thr Met Cys Gln Tyr Thr Ser Pro Thr Pro Gly Pro Met Cys Leu Glu
            20                  25                  30

Tyr Ser Asn Val Gly Phe Thr Asp Ala Glu Lys Asp Ala Ile Val Asn
        35                  40                  45

Lys His Asn Glu Leu Arg Gln Arg Val Ala Ser Gly Lys Glu Met Arg
    50                  55                  60

Gly Thr Asn Gly Pro Gln Pro Pro Ala Val Lys Met Pro Asn Leu Thr
65                  70                  75                  80

Trp Asp Pro Glu Leu Ala Thr Ile Ala Gln Arg Trp Ala Asn Gln Cys
                85                  90                  95

Thr Phe Glu His Asp Ala Cys Arg Asn Val Glu Arg Phe Ala Val Gly
            100                 105                 110

Gln Asn Ile Ala Ala Thr Ser Ser Ser Gly Lys Asn Lys Ser Thr Leu
        115                 120                 125
```

Ser Asp Met Ile Leu Leu Trp Tyr Asn Glu Val Lys Asp Phe Asp Asn
    130   135   140

Arg Trp Ile Ser Ser Phe Pro Ser Asp Gly Asn Ile Leu Met His Val
145   150   155   160

Gly His Tyr Thr Gln Ile Val Trp Ala Lys Thr Lys Lys Ile Gly Cys
                165   170   175

Gly Arg Ile Met Phe Lys Glu Asp Asn Trp Asn Lys His Tyr Leu Val
            180   185   190

Cys Asn Tyr Gly Pro Ala Gly Asn Val Leu Gly Ala Gln Ile Tyr Glu
        195   200   205

Ile Lys Lys
    210

<210> 81
<211> 204
<212> PRT
<213> Vespula vulgaris

<400> 81

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1   5   10   15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
            20   25   30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
        35   40   45

Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu Glu Thr Arg Gly Asn
    50   55   60

Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn Leu Val Trp Asn
65   70   75   80

Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala Asn Gln Cys Gln Tyr
85 90 95

Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr Gln Val Gly Gln Asn
100 105 110

Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr Asp Asp Pro Val Lys
115 120 125

Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp Tyr Asn Pro Lys Lys
130 135 140

Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly His Tyr Thr Gln Met
145 150 155 160

Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly Ser Ile Lys Tyr Ile
165 170 175

Gln Glu Lys Trp His Lys His Tyr Leu Val Cys Asn Tyr Gly Pro Ser
180 185 190

Gly Asn Phe Met Asn Glu Glu Leu Tyr Gln Thr Lys
195 200

<210> 82
<211> 212
<212> PRT
<213> Solenopsis invicta

<400> 82

Thr Asn Tyr Cys Asn Leu Gln Ser Cys Lys Arg Asn Asn Ala Ile His
1 5 10 15

Thr Met Cys Gln Tyr Thr Ser Pro Thr Pro Gly Pro Met Cys Leu Glu
20 25 30

Tyr Ser Asn Val Gly Phe Thr Asp Ala Glu Lys Asp Ala Ile Val Asn

35 40 45

Lys His Asn Glu Leu Arg Gln Arg Val Ala Ser Gly Lys Glu Met Arg
50 55 60

Gly Thr Asn Gly Pro Gln Pro Pro Ala Val Lys Met Pro Asn Leu Thr
65 70 75 80

Trp Asp Pro Glu Leu Ala Thr Ile Ala Gln Arg Trp Ala Asn Gln Cys
85 90 95

Thr Phe Glu His Asp Ala Cys Arg Asn Val Glu Arg Phe Ala Val Gly
100 105 110

Gln Asn Ile Ala Ala Thr Ser Ser Ser Gly Lys Asn Lys Ser Thr Pro
115 120 125

Asn Glu Met Ile Leu Leu Trp Tyr Asn Glu Val Lys Asp Phe Asp Asn
130 135 140

Arg Trp Ile Ser Ser Phe Pro Ser Asp Asp Asn Ile Leu Met Lys Val
145 150 155 160

Glu His Tyr Thr Gln Ile Val Trp Ala Lys Thr Ser Lys Ile Gly Cys
165 170 175

Ala Arg Ile Met Phe Lys Glu Pro Asp Asn Trp Thr Lys His Tyr Leu
180 185 190

Val Cys Asn Tyr Gly Pro Ala Gly Asn Val Leu Gly Ala Pro Ile Tyr
195 200 205

Glu Ile Lys Lys
210

<210> 83
<211> 136
<212> PRT

<213> Lycopersicon esculentum

<400> 83

Gln Asn Ser Pro Gln Asp Tyr Leu Ala Val His Asn Asp Ala Arg Ala
1 5 10 15

Gln Val Gly Val Gly Pro Met Ser Trp Asp Ala Asn Leu Ala Ser Arg
20 25 30

Ala Gln Asn Tyr Ala Asn Ser Arg Ala Gly Asp Cys Asn Leu Ile His
35 40 45

Ser Gly Ala Gly Glu Asn Leu Ala Lys Gly Gly Gly Asp Phe Thr Gly
50 55 60

Arg Ala Ala Val Gln Leu Trp Val Ser Glu Arg Pro Ser Tyr Asn Tyr
65 70 75 80

Ala Thr Asn Gln Cys Val Gly Gly Lys Lys Cys Arg His Tyr Thr Gln
85 90 95

Val Val Trp Arg Asn Ser Val Arg Leu Gly Cys Gly Arg Ala Arg Cys
100 105 110

Asn Asn Asn Gly Trp Trp Phe Ile Ser Cys Asn Tyr Asp Pro Val Gly
115 120 125

Asn Trp Ile Gly Gln Arg Pro Tyr
130 135

<210> 84
<211> 187
<212> PRT
<213> Schizophyllum commune

<400> 84

Ser Pro Ala Pro Val Asp Val Asp Ala Arg Ala Pro Val Ala Leu Asp
1 5 10 15

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Ser Arg Ser Ile Asp Ile Asp Ser Arg Ser Ala Asp Ala Leu Ala Asn
            20                  25                  30

Arg Ala Ala Pro Pro Gln Ser Glu Ile Asp Gln Trp Leu Lys Ala His
        35                  40                  45

Asn Asn Glu Arg Ala Gln His Gly Ala Val Ala Leu Val Trp Asn Gln
    50                  55                  60

Thr Leu Ser Asp Lys Ala Ala Asp Trp Ala Ser Gln Cys Ile Trp Glu
65                  70                  75                  80

His Ser Asn Ser Gly Gln Asn Leu Ala Ala Trp Phe Ser Pro Gln Ala
                85                  90                  95

Asn Lys Pro Met Asn Ile Ser Gln Gly Val Gly Gly Trp Asn Ala Glu
            100                 105                 110

Glu Pro Asp Tyr Asn Thr Thr Thr Tyr Ser Gly Ala Gly His Trp Thr
        115                 120                 125

Gln Val Val Trp Lys Ser Thr Thr Ser Val Gly Cys Ala Ala Tyr Ser
    130                 135                 140

Cys Pro Pro Gly Thr Leu Gly Arg Lys Pro Thr Asp Pro Trp Lys Thr
145                 150                 155                 160

Leu Trp Tyr Tyr Val Cys Asn Tyr Tyr Arg Pro Gly Asn Val Ser Pro
                165                 170                 175

Arg Asp Lys Tyr Tyr Pro Ile Asn Val Gln Pro
            180                 185
```

<210> 85
<211> 239
<212> PRT
<213> Homo sapiens

<400> 85

Ser Thr Val Val Leu Leu Asn Ser Thr Asp Ser Ser Pro Pro Thr Asn
1 5 10 15

Asn Phe Thr Asp Ile Glu Ala Ala Leu Lys Ala Gln Leu Asp Ser Ala
20 25 30

Asp Ile Pro Lys Ala Arg Arg Lys Arg Tyr Ile Ser Gln Asn Asp Met
35 40 45

Ile Ala Ile Leu Asp Tyr His Asn Gln Val Arg Gly Lys Val Phe Pro
50 55 60

Pro Ala Ala Asn Met Glu Tyr Met Val Trp Asp Glu Asn Leu Ala Lys
65 70 75 80

Ser Ala Glu Ala Trp Ala Ala Thr Cys Ile Trp Asp His Gly Pro Ser
85 90 95

Tyr Leu Leu Arg Phe Leu Gly Gln Asn Leu Ser Val Arg Thr Gly Arg
100 105 110

Tyr Arg Ser Ile Leu Gln Leu Val Lys Pro Trp Tyr Asp Glu Val Lys
115 120 125

Asp Tyr Ala Phe Pro Tyr Pro Gln Asp Cys Asn Pro Arg Cys Pro Met
130 135 140

Arg Cys Phe Gly Pro Met Cys Thr His Tyr Thr Gln Met Val Trp Ala
145 150 155 160

Thr Ser Asn Arg Ile Gly Cys Ala Ile His Thr Cys Gln Asn Met Asn
165 170 175

Val Trp Gly Ser Val Trp Arg Arg Ala Val Tyr Leu Val Cys Asn Tyr
180 185 190

Ala Pro Lys Gly Asn Trp Ile Gly Glu Ala Pro Tyr Lys Val Gly Val
195 200 205

Pro Cys Ser Ser Cys Pro Pro Ser Tyr Gly Gly Ser Cys Thr Asp Asn
210 215 220

Leu Cys Phe Pro Gly Val Thr Ser Asn Tyr Leu Tyr Trp Phe Lys
225 230 235

<210> 86
<211> 245
<212> PRT
<213> Homo sapiens

<400> 86

Ala Asn Ile Leu Pro Asp Ile Glu Asn Glu Asp Phe Ile Lys Asp Cys
1 5 10 15

Val Arg Ile His Asn Lys Phe Arg Ser Glu Val Lys Pro Thr Ala Ser
20 25 30

Asp Met Leu Tyr Met Thr Trp Asp Pro Ala Leu Ala Gln Ile Ala Lys
35 40 45

Ala Trp Ala Ser Asn Cys Gln Phe Ser His Asn Thr Arg Leu Lys Pro
50 55 60

Pro His Lys Leu His Pro Asn Phe Thr Ser Leu Gly Glu Asn Ile Trp
65 70 75 80

Thr Gly Ser Val Pro Ile Phe Ser Val Ser Ser Ala Ile Thr Asn Trp
85 90 95

Tyr Asp Glu Ile Gln Asp Tyr Asp Phe Lys Thr Arg Ile Cys Lys Lys
100 105 110

Val Cys Gly His Tyr Thr Gln Val Val Trp Ala Asp Ser Tyr Lys Val
115 120 125

Gly Cys Ala Val Gln Phe Cys Pro Lys Val Ser Gly Phe Asp Ala Leu
    130   135   140

Ser Asn Gly Ala His Phe Ile Cys Asn Tyr Gly Pro Gly Gly Asn Tyr
145   150   155   160

Pro Thr Trp Pro Tyr Lys Arg Gly Ala Thr Cys Ser Ala Cys Pro Asn
                165   170   175

Asn Asp Lys Cys Leu Asp Asn Leu Cys Val Asn Arg Gln Arg Asp Gln
            180   185   190

Val Lys Arg Tyr Tyr Ser Val Val Tyr Pro Gly Trp Pro Ile Tyr Pro
        195   200   205

Arg Asn Arg Tyr Thr Ser Leu Phe Leu Ile Val Asn Ser Val Ile Leu
    210   215   220

Ile Leu Ser Val Ile Ile Thr Ile Leu Val Gln Leu Lys Tyr Pro Asn
225   230   235   240

Leu Val Leu Leu Asp
                245

<210> 87
<211> 223
<212> PRT
<213> Heloderma horridum

<400> 87

Glu Ala Ser Pro Lys Leu Pro Gly Leu Met Thr Ser Asn Pro Asp Gln
1   5   10   15

Gln Thr Glu Ile Thr Asp Lys His Asn Asn Leu Arg Arg Ile Val Glu
            20   25   30

Pro Thr Ala Ser Asn Met Leu Lys Met Thr Trp Ser Asn Lys Ile Ala
        35   40   45

```
Gln Asn Ala Gln Arg Ser Ala Asn Gln Cys Thr Leu Glu His Thr Ser
    50                  55                  60

Lys Glu Glu Arg Thr Ile Asp Gly Val Glu Cys Gly Glu Asn Leu Phe
65                  70                  75                  80

Phe Ser Ser Ala Pro Tyr Thr Trp Ser Tyr Ala Ile Gln Asn Trp Phe
                85                  90                  95

Asp Glu Arg Lys Tyr Phe Arg Phe Asn Tyr Gly Pro Thr Ala Gln Asn
            100                 105                 110

Val Met Ile Gly His Tyr Thr Gln Val Val Trp Tyr Arg Ser Tyr Glu
        115                 120                 125

Leu Gly Cys Ala Ile Ala Tyr Cys Pro Asp Gln Pro Thr Tyr Lys Tyr
    130                 135                 140

Tyr Gln Val Cys Gln Tyr Cys Pro Gly Gly Asn Ile Arg Ser Arg Lys
145                 150                 155                 160

Tyr Thr Pro Tyr Ser Ile Gly Pro Pro Cys Gly Asp Cys Pro Asp Ala
                165                 170                 175

Cys Asp Asn Gly Leu Cys Thr Asn Pro Cys Lys Gln Asn Asp Val Tyr
            180                 185                 190

Asn Asn Cys Pro Asp Leu Lys Lys Gln Val Gly Cys Gly His Pro Ile
        195                 200                 205

Met Lys Asp Cys Met Ala Thr Cys Lys Cys Leu Thr Glu Ile Lys
    210                 215                 220
```

<210> 88
<211> 222
<212> PRT
<213> Homo sapiens

<400> 88

```
Lys Asp Pro Ala Phe Thr Ala Leu Leu Thr Thr Gln Leu Gln Val Gln
1               5                   10                  15

Arg Glu Ile Val Asn Lys His Asn Glu Leu Arg Lys Ala Val Ser Pro
            20                  25                  30

Pro Ala Ser Asn Met Leu Lys Met Glu Trp Ser Arg Glu Val Thr Thr
        35                  40                  45

Asn Ala Gln Arg Trp Ala Asn Lys Cys Thr Leu Gln His Ser Asp Pro
    50                  55                  60

Glu Asp Arg Lys Thr Ser Thr Arg Cys Gly Glu Asn Leu Tyr Met Ser
65                  70                  75                  80

Ser Asp Pro Thr Ser Trp Ser Ser Ala Ile Gln Ser Trp Tyr Asp Glu
                85                  90                  95

Ile Leu Asp Phe Val Tyr Gly Val Gly Pro Lys Ser Pro Asn Ala Val
            100                 105                 110

Val Gly His Tyr Thr Gln Leu Val Trp Tyr Ser Thr Tyr Gln Val Gly
        115                 120                 125

Cys Gly Ile Ala Tyr Cys Pro Asn Gln Asp Ser Leu Lys Tyr Tyr Tyr
    130                 135                 140

Val Cys Gln Tyr Cys Pro Ala Gly Asn Asn Met Asn Arg Lys Asn Thr
145                 150                 155                 160

Pro Tyr Gln Gln Gly Thr Pro Cys Ala Gly Cys Pro Asp Asp Cys Asp
                165                 170                 175

Lys Gly Leu Cys Thr Asn Ser Cys Gln Tyr Gln Asp Leu Leu Ser Asn
            180                 185                 190
```

Cys Asp Ser Leu Lys Asn Thr Ala Gly Cys Glu His Glu Leu Leu Lys
195 200 205

Glu Lys Cys Lys Ala Thr Cys Leu Cys Glu Asn Lys Ile Tyr
210 215 220

<210> 89
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide

<400> 89

Glu Ala Glu Ala Glu Phe
1 5

<210> 90
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide

<400> 90

Glu Ala Glu Phe
1

<210> 91
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide

<400> 91

Arg Glu Ala Glu Ala Glu Phe
1 5

<210> 92
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide

<400> 92

Glu Glu Gly Val Ser Leu Glu Lys Arg
1 5

<210> 93
<211> 50
<212> PRT
<213> Vespula vulgaris

<400> 93

Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly Gly Val His Thr Ala
1 5 10 15

Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly Asn Lys Val Val Val
20 25 30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
35 40 45

Asn Asp
50

<210> 94
<211> 57
<212> PRT
<213> Vespula vulgaris

<400> 94

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Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
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Asn Asp Phe Arg Gln Lys Ile Ala Arg
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20 25 30

Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp Ile Leu Lys Glu His
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Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys Asn
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